1221913

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

September 07, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/491,553*
FILING DATE: *August 01, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/24760*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

PTO/SB/16 (10-01)
Approved for use through10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. Fedex 8292 8191 7456

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
JOHN M.	NEWSAM	SAN DIEGO, CALIFORNIA
ILYA	FEYGIN	MOUNTAINSIDE, NEW JERSEY
SAMIR	MITRAGOTRI	GOLETA, CALIFORNIA

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

RAPID ASSAYING OF FORMULATIONS APPLIED TO SKIN

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*

OR *Type Customer Number here*

☒ Firm or Individual Name	HTE NORTH AMERICA, DBA 'FQUBED'				
Address	6540 LUSK BOULEVARD, SUITE C276				
Address					
City	SAN DIEGO	State	CALIFORNIA	ZIP	92121
Country	USA	Telephone	(858) 677-6733	Fax	(858) 677-6737

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 30
☒ Drawing(s) *Number of Sheets* 14
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☐ Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

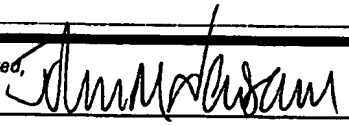
☒ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fees
☐ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number:
☒ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE 

Date 07/31/03

TYPED or PRINTED NAME JOHN M. NEWSAM

REGISTRATION NO. *(if appropriate)*

Docket Number: 113-003US

TELEPHONE (858) 677-6733

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

The stamp of the USPTO acknowledging receipt
is requested.

---

(1) Prov. Appl. For Patent Cover Sheet (1 pg)
(2) Credit Card Payment Form (1 pg)
(3) Disclosure –
    a. 30 pgs of Description
    b. 14 pgs of Drawings

113-003US

Rapid Assaying of Formulations Applied to Skin

ABSTRACT

The present invention teaches devices and methods for screening, in high throughput, the interaction of formulations with skin, that are especially beneficial when applied in an array format. The devices and methods enable more efficient measurements of permeation through skin, and of the absorption and adsorption by skin of ingredients in fluid formulations, together with screening of exfoliation of material from the exterior of the stratum corneum and of dermal irritation by such ingredients. The devices provide for fluid contact to the skin from both donor and receptor sides, for measurements of skin electrical response in the presence of formulations, of permeation and permeation enhancement, for the depth-profiling of formulation constituents through the skin, of dermal irritation, of stratum corneum component disruption, and of loss of material from the stratum corneum. The invention thus provides simple, robust and scalable means of performing, preferably in high throughput, studies of transdermal drug delivery, transdermal delivery of proteins or DNA, and the development of personal care products, such as cosmetics, moisturizers and exfoliants.

FIELD OF THE INVENTION

The present invention relates generally to the development of chemical or biological agents for application, in formulations, to or through skin, and more particularly to rapid and efficient methods of measuring both permeation of such agents into and through skin, and other properties associated with their interactions with skin.

BACKGROUND OF THE INVENTION

Skin and Formulations Applied to Skin

The skin, the largest organ of the human body, has three layers - the epidermis, dermis and subcutis. The subcutis, the deepest layer, provides thermal insulation and has a shock-absorbing effect that helps protect the body's organs from injury. The dermis, the middle layer, contains hair shafts, sweat glands, blood vessels and nerves. The top layer of the skin is the epidermis, separated from the other layers of skin by the basement membrane which serves as the "glue" at the dermal - epidermal junction. The epidermis is relatively thin, and it is divided into four layers, from the innermost to outermost: the basal cell layer, stratum spinosum, stratum granulosum, and stratum corneum. The basal cell layer contains basal cells which divide and differentiate into other cells in the epidermis, and melanocytes, the cells that make melanin which gives skin its color. The stratum spinosum lies outside the basal cell layer and is comprised of keratinocytes, cells that make the protein keratin, an important component of the stratum corneum as well as of hair and nails. Cells in the stratum granulosum are flattened and contain dark granules that are expelled and provide the "cement" that holds cells together in the overlying stratum corneum. The stratum corneum, the outermost layer of the epidermis,

is comprised of tightly-packed layers of dead cells filled with keratin. It forms the major physical barrier for the skin. The stratum corneum is thicker in areas like the palms and soles that withstand more daily wear and tear than those of other parts of the body. The epidermis also contains Langerhans cells, which act as part of the skin's defense against infection.

While skin serves as the body's natural barrier against incursion of chemical or pathogenic factors, it also has substantial appeal as a route of introduction of small molecule drugs, as an alternative to oral, buccal, pulmonary or other parenteral routes. The prime limitation to transdermal drug delivery is skin's low permeability. Sonophoretic, electroporative and other physical treatments that enhance permeation show promise, but a simple chemical means of selectively enhancing the skin permeation of a given drug, that has minimum collateral effects and a good safety profile, is highly desired. Some 200 different chemical permeation enhancers have already been considered, including surfactants, fatty acids, fatty alcohols, and organic solvents. However, very few are useful in a practical sense – many of these entities do not provide a significant enhancement of transdermal drug permeation, and several cause skin irritation or present other safety issues. Given that there are several mechanisms by which drug permeation through skin might be enhanced, the combined use of two or more permeation enhancers, each potentially at low concentration, might generate substantially increased permeability, while reducing irritation or safety concerns. Limited data on chemical enhancer combinations indeed show that a combination of two or more enhancers can be more effective in increasing transdermal transport compared to either alone. However, the number of possible combinations of even the already known 200 prospective enhancers is vast. Further, our understanding of the underlying molecular mechanisms is quite limited, and the performance of any given combination cannot today be predicted. In order to develop effective permeation enhancer combinations, therefore, what is needed is an efficient experimental means of producing such combinations and screening the degree to which they enhance the permeation of the given drug, as well as the degree to which they cause irritation or other skin damage.

Skin also has nascent potential as a route for the delivery of proteins [1] and of genes into the body. In order to develop chemical agents that are effective at promoting the permeation through skin of proteins or of deoxyribonucleic acid ("DNA"), what is again needed is an efficient experimental means of screening large numbers of combinations of such agents and many different packagings of the proteins or nucleic acids, for their effectiveness at achieving effective transport, without causing irritation or other skin damage.

The personal care domain is a third major sector that is concerned with the interaction of formulations with skin. Personal care products, that include cosmetics, lotions, salves, creams, moisturizers, exfoliants, cleansers or colorants, improve the health, the feel or the appearance of skin.

Emollients, which soften skin, and moisturizers, which add moisture, are used to correct dryness and scaling of the skin. Dry skin results from loss of water from the stratum corneum, causing it to lose its flexibility and become cracked and scaly. The stratum

corneum contains natural water-holding substances that retain water seeping out from the deeper layers of the skin. Water is also retained in the stratum corneum by a surface film of natural oil (sebum) and broken-down skin cells, which hinder trans-epidermal water loss ("TEWL") through evaporation. Moisturizers and emollients can function through one or both of two actions [2]. Occlusives provide a layer of oil on the surface of the skin that slows water loss and thus increases the moisture content of the stratum corneum. Humectants are substances, exemplified by glycerin, urea or alpha hydroxy acids [3] such as lactic acid or glycolic acid, that, when introduced into the stratum corneum, increase its water holding capacity.

An exfoliant, or peeling agent, acts to slough away dead epidermal skin cells and encourage accelerated cell renewal, thus promoting soft and smooth skin that has visual appeal. Exfoliants function by promoting thinning of the stratum corneum through a descaling or keratolytic action.

To develop formulations that are effective as moisturizers, emollients or exfoliants, to assess the impact on skin of other topical applications such as cosmetics, sun screens, salves and cleansers, and to evaluate the biological impact of prospective active ingredients in cosmetics, as in cosmeceuticals, what is again needed is an efficient experimental means of screening large numbers of such formulations for their ability to do one or more of: (i) adsorb to the outer skin surface, (ii) be absorbed into the stratum corneum or other skin layers, (iii) permeate through the stratum corneum, (iv) permeate through the other skin layers and into the vasculature, (v) cause epidermal irritation, and (vi) alter the optical characteristics of skin by other mechanisms.

Skin Permeation Studies

The traditional method of performing skin permeation studies, including of topical and transdermal drug delivery formulations as well as of ophthalmics, cosmetics, skin care products and pesticides, employs a vertical diffusion cell, first described by T. Franz. Permeation of a chemical agent from an upper donor well, through a skin sample, into a lower receptor well is assessed, under steady state conditions, through analysis of the concentration of chemical agent in the donor and receptor wells, typically by high performance liquid chromatography ("HPLC"). A single Franz diffusion cell can typically perform about one test per square inch of skin per day. An automated Franz diffusion cell - HPLC system with 6 cells is available from Logan Instruments Corporation of Somerset, NJ. This method, though useful for quantifying the drug dose delivered across the skin, is not suitable for high throughput screening as it (i) requires a relatively large area of skin, (ii) requires a substantial equilibration time to reach the steady state, and (iii) requires substantial manual operations associated with sample collection and handling.

Discrete designs different from the Franz diffusion cell have also been disclosed, including Bronaugh's Flow Through Diffusion cell and Moody's AIVDA system; these also operate on the same principle of steady-state flux measurements and are being used for trans-dermal testing. Despite their claimed advantages over Franz diffusion cells, however, their efficiency in screening enhancers is similar to that with Franz diffusion cells.

A related device used to measure the flow of metabolites across a membrane is the Ussing chamber, originally developed to measure the passage of water and sodium ions across short-circuited, isolated frog skin. Like the Franz diffusion cell, the Ussing Chamber consists of an upper donor chamber and a lower receptor chamber, with passage of a chemical agent through the membrane that separates the chambers being measured by analysis of the receptor well contents as described in a paper by Ussing [4], which is incorporated herein by reference. It differs, though, in being equipped to circulate and aerate the buffer solutions on donor and receptor sides, and to measure also the electrical potential across the membrane. Individual Ussing chambers are available, for example, from World Precision Instruments, of Sarasota, FL. A 6-fold Ussing chamber arrangement is available from Dipl.-Ing. K. Mussler Scientific Instruments, of Aachen, Germany. Ussing chambers or modified Ussing chambers (e.g. [5]) have been used extensively to measure ion and metabolite transport across many types of membrane but, like the Franz diffusion cell, the Ussing chamber is unsuitable for use in high throughput screening.

An alternative to these discrete cell designs is to use an array format. Thus, US Patent 5,490,415, which is incorporated herein by reference, teaches an apparatus used to test diffusion of a drug through a test membrane in which a number of open-top receptor vessels addresses a test membrane captured between this receptor vessel array and a mirror-image donor vessel array. The drug diffuses from a given donor well through the test membrane and into the receptor liquid in the corresponding receptor well. Samples of the receptor liquid might then be transferred using a programmed liquid transfer system, perhaps for assay by a scintillation counter [6]. Similarly, US Patent 6,043,027, which is incorporated herein by reference, teaches testing devices, systems, and methods for evaluating the permeation of various chemicals through different types of cells. One such device is described to comprise a base member and a top member having a plurality of wells which are aligned when the top member is secured to the base member. A membrane sheet which includes at least one layer of cells grown on the sheet is placed between the base member and the top member prior to assembly. Test samples are placed into the wells in the top member and samples are removed from the top and bottom wells at a later time and tested to determine the amount of test sample which permeated through the cells [7].

Still more recently, WO 02/06518 A1, which is incorporated herein by reference, claims an apparatus for measuring transfer of components across a tissue, comprising a support plate; an array of samples supported by the support plate; a tissue specimen overlaying the array of samples; and a reservoir plate secured to a side of the tissue specimen opposite the array of samples, the reservoir plate having an array of reservoirs [8].

What is termed a combinatorial method for rapid screening of drug delivery formulations has been disclosed in papers by Mitragotri et al. [9,10] , both of which are incorporated herein by reference. This method teaches the use of an array of wells, each potentially containing a different formulation, applied to a single piece of skin, with permeation being monitored via quantitative changes in the single point conductivity of the stratum corneum in the vicinity of each well. Although measurement of drug penetration into the skin directly measures the increase in drug delivery, the skin conductivity measurement

provides an indirect but rapid assay to determine the effect of enhancers on skin permeability, especially for polar drugs [10].

The conductivity measurements are calibrated by comparison with direct permeation measurements, either in the same experimental set-up or in Franz diffusion cells operated under similar conditions [10]. This method does provide significant gains in the speed with which permeation measurements can be made, but it is not suitable for making measurements at short contact times, has potential problems with incomplete contact of donor and receptor fluids with skin due to the presence of bubbles, does not allow for partial or complete inversion of the apparatus, and does not support measurements of skin properties other than permeation.

Use of an Array Format

As the methods and devices of the present invention are of particular benefit when applied in an array format, the general features of array-based membrane permeation measurement systems are described in more detail. FIG. 1 shows a schematic diagram of the base components of such an array system. The base elements of the system comprise a donor plate supporting a donor sample array, a lamina, and a receptor plate.

The donor plate may be any rigid grid or plate capable of supporting a number of samples. For example, the donor plate may be a 24, 36, 48, 72, 96 or 384 well plate. Preferably, the size of a sample well is about 1 mm to about 50 mm, more preferably about 2 mm to about 10 mm, and most preferably about 3 mm to about 7 mm. For example, a 3 mm well format provides an array of approximately 30,000 samples for 0.25 m^2 of skin. An array can comprise 24, 36, 48, 96, or more samples, preferably at least 1,000 samples, more preferably, at least 10,000 samples. An array is typically comprised of one or more sub-arrays. For example, a sub-array can be a plate having 96 sample wells.

To the bottom of the donor plate is attached a base that forms the bottom of each donor well. The base is optionally a membrane made of any suitable material (e.g. a rubber or Teflon membrane) in a fashion that permits air to bleed out of the sample well when it is being filled with a sample. The base may also be a rigid, removable substrate plate.

Between donor and receptor wells is placed a lamina. The lamina may be a contiguous piece of tissue, such as a skin, as shown in the figure. Alternatively, the lamina may be cut into a plurality of segments or strips. Cuts are beneficially applied to prevent lateral diffusion through the tissue specimen between adjacent samples wells, and to improve the electrical isolation of adjacent samples. Cuts through the lamina may be made in any number of ways, including mechanical scribing or cutting, laser cutting, or crimping. Preferably, laser scribing is used as it avoids mechanical pressure from a cutting tool which can cause distortion and damage to the lamina. Laser cuts are performed with very small kerfs which permit a relatively high density of samples and a more efficient tissue specimen utilization. Laser tools are available that minimize the region that is heated, thereby reducing damage to tissue specimen.

A receptor plate, such as an open-bottomed titer plate, is placed on the side of the lamina opposite to the donor plate. The receptor plate may comprise a single common reservoir, or an array of reservoirs that mirrors, in well layout, that of the donor plate.

The donor plate, receptor plate and lamina are assembled as illustrated. The receptor plate is secured to the donor plate using clamps, screws, fasteners, or other suitable attachment means, with sufficient pressure so as to create a liquid-tight seal around the reservoirs in both donor and receptor plates. Each well is loaded with a solvent, solution or sample, such that sample components or compounds diffuse across the lamina from the donor wells to the receptor well or wells. This transfer or flux of components from donor wells, the tissue barrier transfer, diffusion or permeation, may be analyzed by measuring component concentration in specimens taken from receptor well or wells.

Use of an array format, in addition to compactness, has the advantage that various automated methods of introduction samples are readily applied. Various automated distribution systems for simple liquids are commercially available, such as the MultiPROBE® II and MultiPROBE® EX, available from PerkinElmer Life and Analytical Sciences, Inc. of Boston, MA (las.perkinelmer.com), the Multiple Probe 215 and Constellation™ 1200 available from Gilson, Inc. of Middleton, WI (www.gilson.com), the Microlab STAR available from Hamilton Company of Reno, NV (www.hamiltoncomp.com), the synQUAD available from Genomic Solutions (Cartesian Technologies) of Irvine CA (www.cartesiantech.com), the Tango™ available from Matrix Technologies Corp. (Robbins Scientific) of Sunnyvale CA (www.robsci.com), and the Genesis and Genesis NPS, available from Tecan, headquartered in Männedorf near Zurich, Switzerland (www.tecan.com).

Critical Unmet Needs

A system suitable for the efficient screening of the permeation through skin and the influence of active ingredients in formulations upon skin would thus desirably have the following characteristics:

(1) able to accommodate measurements on skin, as well as on other biological membranes, certain of which might require special conditions to maintain viability such as aeration or irrigation;
(2) require minimal amount of skin (or other membrane) and reagents per measurement;
(3) be efficient in throughput, preferably capable of making thousands of separate measurements per day, and hence necessarily being compatible with the various technologies, including automation, robotics, experiment and data management systems, that are aspects of a high throughput experimentation approach;
(4) support measurements of each of the following properties, preferably all as part of a single experimental set-up:
    (a) **Electrical response:** of the stratum corneum as function of exposure to the agent(s) in the formulation. The conductivity measurement is also a direct indication of the skin quality – if the conductivity is below a threshold, then the skin sample can be considered spoiled. Similarly, the conductivity measurement can reveal problems with the experimental set-up (such as bubbles at the skin-formulation interface). In addition to a single point conductivity measure, a more detailed analysis of the temporal response to a momentary voltage pulse is desirable.

(b) **Permeation:** while a correlation between the conductivity and permeability that is approximately linear is generally seen, the gradient and intercept values differ, in currently unpredictable ways, from one permeating molecule to the next. Hence an ability to also make direct measurements of permeation is preferred.

(c) **Irritation:** the degree of skin irritation is a key concern in both pharmaceutical and personal care domains, even if the time during which the formulation is in contact with the skin may vary substantially from one application to another. Skin irritation derives primarily from damage to epidermal cells, the lysed contents of which then trigger an inflammation response. Irritation may thus be deduced from the proportion of epidermal cells that is viable. This can be assayed using MTT or XTT, tetrazolium salts which are reduced by succinate dehydrogenase within the mitochondria of metabolically active cells to form a colored formazan dye. (MTT is (3-(4,5-dimethylthiazol-2yl)-2,5 diphenyltetrazolium bromide) and XTT is (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide). The proportion of viable epidermal cells is deduced from the rate of change in absorbance, expressed as the ratio of the optical densities at 540nm and 690nm.

(d) **Absorption:** the degree to which a molecule or material is taken up by the stratum corneum, but without permeation through the entire epidermis is a prime question in development of moisturizers, emollients or adhesives.

(e) **Adsorption:** the adherence of molecular or other entities to the skin exterior is of interest in testing and development of cosmetics or occlusive moisturizers.

(f) **Exfoliation:** quantifying the extent to which the sloughing off of material from the stratum corneum is facilitated by agents in a formulation is a prime question in emollient development.

While the optimal system might support measurements of all of these types in a high throughput, low information content mode, a single primary property measure might also be used as a primary filter. In many cases, for example in development of skin creams or transdermally delivered drugs, conductivity might be used as a primary filter. A certain permeation threshold, signaled as a conductivity threshold, might be set, as low permeation formulations can be discarded. Those formulations that pass this primary conductivity test might next be evaluated for irritation. Those that then pass the irritation test might then be examined in still further detail, perhaps from a fundamental mechanistic perspective.

For certain types of screening, it will be desirable to add additional property measures to this core list. For example, for live skin samples, the rate of trans-epidermal water loss is often of interest, particularly in development of moisturizers; measures TEWL have been described in papers by Nilsson [11], Tagami [12] and Imhof et al. [13], which are each incorporated herein by reference.

(5) yield data that in each category that are comparable with (or which can be calibrated against or correlated with) those from traditional experiments.

(6) able to accumulate data over a range of times of contact of the formulations with the skin, from several minutes to many hours, and with the possibility of first measurements being accumulated within a few seconds of first contact.

(7) accommodate a range of formulation types, encompassing aqueous or non-aqueous solutions, emulsions and hydrocarbon-based lotions, formulations that might be rubbed onto the skin, and formulations with a volatile component that will evaporate.

(8) support a range of operating conditions, including temperatures from 0°C to 50°C and, on occasion, oxygen-free environments.

SUMMARY OF THE INVENTION

The present invention teaches devices and methods for screening, in high throughput, the interaction of formulations with skin, that are especially beneficial when applied in an array format. The devices and methods enable more efficient measurements of permeation through skin, and of the absorption and adsorption by skin of ingredients in fluid formulations, together with screening of exfoliation of material from the exterior of the stratum corneum and of dermal irritation by such ingredients. The devices and methods use, as a foundation, an array format based on a set of donor vessels, an interleaving piece or pieces of skin, and a set of receptor vessels. The method and devices allow formulations to be prepared, and for all donor wells to be loaded before contact by the formulation with the skin is made, and for this contact to then be initiated essentially simultaneously for all donor wells. A simple device ensures continued fluid contact with the skin sample, independent of the orientation of the device as a whole, as well as providing for the abstraction of samples from the donor well for chemical analysis. Further methods and devices are disclosed for preventing cross-talk between adjacent wells, as well as providing for the removal of gas bubbles or of fluid for analysis. The donor wells may each be provided with an electrode, so that the response of the stratum corneum to a voltage pulse applied through a second electrode mounted into the basal layers or receptor wells can be monitored in an array format.

The invention teaches other methods and devices for the depth-profiling of formulation constituents through the skin, of dermal irritation, of stratum corneum component disruption, and of loss of material from the stratum corneum through exfoliation.

The present invention provides several substantial advantages over the prior art, including (i) experiments can be performed in a vertical geometry, but with the donor cells beneath the skin, (ii) samples can be added or removed from the donor wells during experiments, allowing dynamic measurements of various types to be performed, and (iii) electrical measurements can be made, separately, in all of the wells in a timescale of seconds or less. The present invention also teaches other donor plate designs such as straight-through which, in a donor-cell-uppermost configuration, support measurements of adsorption or exfoliation. The receptor wells are also provided in one of several formats including (i) as a single bath, but provided with posts that provide mechanical support for the skin, and (ii) as an array of receptor wells which mirrors in array layout that of the donor vessel array. The receptor wells can be filled with fluids, for example, phosphate buffer solution ("PBS"), in the same manner in which the donor wells are filled and each can be equipped with the aforementioned device for ensuring fluid contact with the skin irrespective of the orientation of the device as a whole. The device can be mounted within the space that is addressed by a fluid dispensing and aspirating robot. Measurements of epidermal activity, through color development or fluorescence can therefore be automated.

The invention thus provides simple, robust and scalable means of performing, in high throughput, studies of the intra- or trans-dermal delivery, and of several other properties including absorption, adsorption and irritation, of active components of various types, such as small or large molecule drugs, peptides and proteins, DNA, and constituents of personal care products, such as cosmetics, moisturizers and exfoliants. The invention also supports screening.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG 1. A schematic of an exemplary workflow followed to realize benefit from the present method and devices.

FIG 2. Alternative examples of the array well geometries similar to those described in the prior art (FIG. 2A and FIG. 2B). FIG. 2A has an array of receptor wells that mirrors the array layout of the donor wells. FIG. 2B has a single receptor bath, with an array of posts that provide mechanical support for the skin piece(s). Schematics of array well geometries made practical by the present invention are shown in FIG. 2C and FIG. 2D. FIG. 2C illustrates a device assembly with donor wells lowermost and with straight-through receptor wells, a configuration suitable for combined high throughput measurements of both permeation and irritation. FIG. 2D illustrates a device assembly with donor wells uppermost.

FIG 3. Implementation of an array format according to the present invention.

FIG 4. Illustration of clamp-application device used to clamp donor plate – lamina - receptor plate assemblies.

FIG 5. Devices for achieving complete well filling while avoiding the formation of bubbles or air pockets. FIG. 5A illustrates delivery of sample into a well from a syringe, through a canula that pierces a sealing septum. FIG. 5B illustrates delivery through a needle inserted through a duck bill well sealing device.

FIG 6. Duck bill valve devices for complete well filling.

FIG 7. Alternative well sealing systems suitable for allowing for complete well filling with avoidance of air pockets or bubbles.

FIG 8. Illustrates use of a well wall that is partially collapsible in a concertina fashion, suitable for use when specimens are to be extracted from a well.

FIG 9. Illustrates a motor-driven stripping-roller device used for layer-by-layer removal of materials from lamina.

FIG 10. Schematic of typical workflow followed in performing layer-by-layer analysis.

FIG 11. Illustration of device for dispensing samples that are soft materials.

FIG 12. Illustration of application of sonication to an array that contains soft material samples to ensure uniform contact of each sample with the lamina and/or to disrupt the microstructure of the sample(s).

FIG 13. Schematic of electrode arrays applied to an array of donor wells.

FIG 14. Schematic of wiring diagram for an 8 by 12 array of donor wells each with an independent electrode (FIG. 14A) and for bridge technique for accumulating electrical response data (FIG. 14B).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to high throughput systems and methods that improve the efficiency and speed by which formulations suitable for applying active ingredients to tissues, into tissues or through tissues can be discovered, developed and optimized. The systems and methods are suitable for developing formulations suitable for trans-tissue transfer of active compounds, such as pharmaceuticals or drugs, other compounds, or compound combinations. In one embodiment, the systems and methods may be used to identify the optimal components, such as solvents, carriers, transport enhancers, adhesives, additives, and other excipients, for pharmaceutical formulations that are delivered to a patient via tissue transport, including without limitation, pharmaceutical compositions or formulations administered or delivered transdermally (e.g., in the form of a transdermal delivery device), topically (e.g., in the form of ointments, lotions, gels, and solutions), and ocularly (e.g., in the form of a solution). In another embodiment, the systems and methods may be used to identify the optimal components in topically applied skin moisturizers, anti-aging creams, sun screens and other personal care products

Definitions and Terminology

As used herein:

"*active component*" means a substance or compound that imparts a primary utility to a composition or formulation when the composition or formulation is used for its intended purpose. Examples of active components include pharmaceuticals, vitamins, ultra violet ("UV") radiation absorbers, cosmeceuticals, alternative medicines, and nutraceuticals. Active components can optionally be genetic material, such as DNA, diagnostic or sensory compounds, agrochemicals, the active component of a consumer product formulation, or the active component of an industrial product formulation.

"*adhesive*" means an agent used to affix an object, such as a patch, to a tissue, such as skin. The adhesive may form the matrix of such a patch in which an active component is dissolved or dispersed. The compatibility of the active component with an adhesive is influenced by its solubility in that adhesive; a high solubility is desired in the adhesive to increase the driving force for permeation through the tissue and to improve the stability of the device. Classes of polymers used as adhesives include polyisobutylene, silicone, and acrylic adhesives.

"*array*" or "*sample array*" mean a plurality of samples associated under a common experiment.

"*automated*" or "*automatically*" refers to the use of computer software and robotics to achieve one or more experimental operations such as dispensing, weighing, moving, adding, mixing or analyzing objects, samples, components of specimens.

"*component*" means any substance or compound. A component can be active or inactive.

"*excipient*" means the inactive substances used to formulate pharmaceuticals as a result of processing or manufacturing needs, or used by those skilled in the art to formulate pharmaceuticals, alternative medicines, cosmeceuticals, cosmetics, personal care products, dietary supplements, and nutraceuticals for administration to animals or humans.

Preferably, excipients are approved for or considered to be safe for human and animal administration. Examples of suitable excipients include, but are not limited to, acidulents, such as lactic acid, hydrochloric acid, and tartaric acid; solubilizing components, such as non-ionic cationic, and anionic surfactants; absorbents, such as bentonite, cellulose, and kaolin; alkalizing components, such as diethanolamine, potassium citrate, and sodium bicarbonate; anticaking components, such as calcium phosphate tribasic, magnesium trisilicate, and talc; antimicrobial components, such as benzoic acid, sorbic acid, benzyl alcohol, benzethonium chloride, bronopol, alkyl parabens, cetrimide, phenol, phenylmercuric acetate, thimerosol, and phenoxyethanol; antioxidants, such as ascorbic acid, alpha tocopherol, propyl gallate, and sodium metabisulfite; binders, such as acacia, alginic acid, carboxymethyl cellulose, hydroxyethyl cellulose; dextrin, gelatin, guar gum, magnesium aluminum silicate, maltodextrin, povidone, starch, vegetable oil, and zein; buffering components, such as sodium phosphate, malic acid, and potassium citrate; chelating components, such as EDTA, malic acid, and maltol; coating components, such as adjunct sugar, cetyl alcohol, poly-vinyl alcohol, carnauba wax,

lactose maltitol, titanium dioxide; controlled release vehicles, such as microcrystalline wax, white wax, and yellow wax; desiccants, such as calcium sulfate; detergents, such as sodium lauryl sulfate; diluents, such as calcium phosphate, sorbitol, starch, talc, lactitol, polymethacrylates, sodium chloride, and glyceryl palmitostearate; disintegrants, such as colloidal silicon dioxide, croscarmellose sodium, magnesium aluminum silicate, potassium polacrilin, and sodium starch glycolate; dispersing components, such as poloxamer 386, and polyoxyethylene fatty esters (polysorbates); emollients, such as cetearyl alcohol, lanolin, mineral oil, petrolatum, cholesterol, isopropyl myristate, and lecithin; emulsifying components, such as anionic emulsifying wax, monoethanolamine, and medium chain triglycerides; flavoring components, such as ethyl maltol, ethyl vanillin, fumaric acid, malic acid, maltol, and menthol; humectants, such as glycerin, propylene glycol, sorbitol, and triacetin; lubricants, such as calcium stearate, canola oil, glyceryl palmitosterate, magnesium oxide, poloxymer, sodium benzoate, stearic acid, and zinc stearate; solvents, such as alcohols, benzyl phenylformate, vegetable oils, diethyl phthalate, ethyl oleate, glycerol, glycofurol, polyethylene glycol, tartazine, triacetin; stabilizing components, such as cyclodextrins, albumin, xanthan gum; and tonicity components, such as glycerol, dextrose, potassium chloride, and sodium chloride; and mixtures thereof. Excipients include those that alter the rate of absorption, bioavailability, or other pharmacokinetic properties of pharmaceuticals, dietary supplements, alternative medicines, or nutraceuticals. Other examples of suitable excipients, such as binders and fillers are listed in *Remington's Pharmaceutical Sciences, 18th Edition*, Ed. Alfonso Gennaro, Mack Publishing Co. Easton, PA, 1995 and *Handbook of Pharmaceutical Excipients, 3rd Edition*, Ed. Arthur H. Kibbe, American Pharmaceutical Association, Washington D.C. 2000, both of which are incorporated herein by reference. Excipients that are typically used in the formation of transdermal delivery devices, and therefore particularly useful for formulation of the samples of the present invention, are penetration enhancers, adhesives and solvents.

"*high throughput*" refers to the number of samples generated or screened in a given time period as described herein, typically at least 10, more typically at least 50 to 100, and preferably more than 1000 samples. The high throughput experimentation methods of the present invention can be performed using various forms of samples. Typically, the methods are performed either with liquid samples or with solid or semi-solid samples.

"*inactive component*" means a component that is useful or potentially useful to serve in a composition or formulation for administration of an active component, but does not significantly share in the active properties of the active component or give rise to the primary utility for the composition or formulation. Examples of suitable inactive components include, but are not limited to, enhancers, excipients, carriers, binders, solvents, diluents, stabilizers, additives, adhesives, and combinations thereof.

"*lamina*" means a piece of membrane or a sheet of tissue, the interaction of samples with which is tested in the present invention. Examples of suitable types of tissue suitable for use as the lamina include, but are not limited to, skin, lung, tracheal, nasal, placental, vaginal, rectal, colon, gut, stomach, bladder, or corneal tissue. Preferably, stratum corneum or skin tissue, such as hairless mouse skin, porcine skin, guinea pig skin, or human skin is used. If human cadaver skin is to be used,

one known method of preparing the tissue specimen entails heat stripping by keeping it in water at 60°C for two minutes followed by the removal of the epidermis, and storage at 4°C in a humidified chamber; a piece of epidermis is taken out from the humidified chamber prior to the experiments and optionally be supported by a porous support such as Nylon mesh (available from Sefar America Inc. (Tetko Inc.) of Depew, NY; www.sefaramerica.com, or Fisher Scientific of Pittsburgh, PA; www.fishersci.com) to avoid any damage and to mimic the fact that the skin *in vivo* is supported by mechanically strong dermis. Other types of tissues may also be used for the lamina, including living tissue explants, any of a number of endothelial or epithelial cell culture barriers, such as those described in Audus, K. L., et al., *Pharmaceutical Research*, 1990, 7 (5), p 435, animal tissue (e.g. rodent, bovine or swine) or engineered tissue-equivalents. Examples of suitable engineered tissues include DERMAGRAFT®, a human fibroblast-derived dermal substitute (available from Smith & Nephew, Inc. of Largo FL; www.dermagraft.com), and EpiDerm™ a skin model from human-derived epidermal keratinocytes available from MatTek Corporation, Ashland, MA (www.mattek.com), and those taught in U.S. Patent No. 5,266,480, which is incorporated herein by reference. A synthetic membrane, such as an elastomeric membrane, may also be used. The membrane or tissue used as the lamina is chosen based in the desired application. Screening of formulations for transdermal delivery is preferably conducted using pigskin; to screen formulations for oral drug delivery mucosal membrane might be used, and so forth.

"*liquid form*" means that the sample containing the component or components being measured or analyzed is in the form of a liquid, which includes, without limitation, liquids, solutions, emulsions, suspensions, and any of the foregoing having solid particulates dispersed therein.

"*permeation enhancer*" or, equivalently, "*penetration enhancer*" means a substance used to modify, usually to increase, the rate of permeation through skin or other tissue of one or more components in a formulation. Over 250 enhancers have been used for enhancing transdermal drug transport, some of which are listed below. These enhancers are compiled from over 350 references and have been classified into several categories and subcategories based on their structure or their effect on permeability:

Surfactants: These are amphiphilic molecules with a hydrophilic head and a hydrophobic tail group. The tail length and the chemistry of the head group play an important role in determining their effect on skin permeability. Surfactants can be categorized into four groups, cationic, anionic, non-ionic, and zwitterionic depending on the charge on the head group. Prominent examples of surfactants that have been used for transdermal delivery include: Brij (various chain lengths), HCO-60 surfactant, Hydroxypolyethoxydodecane, Lauryl sarcosine, Nonionic surface active agents, Nonoxynol, Octoxynol, Phenylsulfonate, Pluronic, Polyoleates (nonionic surfactants) Rewopal HV10, Sodium laurate, Sodium oleate, Sorbitan dilaurate, Sorbitan dioleate, Sorbitan monolaurate, Sorbitan monooleates, Sorbitan trilaurate, Sorbitan trioleate, Span 20, Span 40, Span 85, Synperonic NP, Triton X-100, Tweens, Sodium alkyl sulfates, and alkyl ammonium halides.

Azone and related compounds: These compounds are also amphiphilic and possess a nitrogen molecule in their head group (preferably in the ring). The presence of a nitrogen atom in a ring creates a bulky polar head group with the potential for strong disruption of stratum corneum. Examples of such compounds include N-Acyl-hexahydro-2-oxo-1H-azepines, N-Alkyl-dihydro-1,4-oxazepine-5,7-diones, N-Alkylmorpholine-2,3-diones, N-Alkylmorpholine-3,5-diones, Azacycloalkane derivatives (-ketone, -thione), Azacycloalkenone derivatives, 1-[2-(Decylthio)ethyl]azacyclopentan-2-one (HPE-101), N-(2,2), Dihydroxyethyl) dodecylamine, 1-Dodecanoylhexahydro-1-H-azepine, 1-Dodecyl azacycloheptan-2-one (azone or laurocapram), N-Dodecyl diethanolamine, N-Dodecyl-hexahydro-2-thio-1H-azepine, N-Dodecyl-N-(2-methoxyethyl)acetamide, N-Dodecyl-N-(2-methoxyethyl) isobutyramide, N-Dodecyl-piperidine-2-thione, N-Dodecyl-2-piperidinone, N-Dodecyl pyrrolidine-3,5-dione N-Dodecyl pyrrolidine-2-thione, N-Dodecyl-2-pyrrolidone, 1-Farnesylazacycloheptan-2-one, 1-Farnesylazacyclopentan-2-one, 1-Geranyl azacycloheptan-2-one, 1, Geranylazacyclopentan-2-one, Hexahydro-2-oxo-azepine-1-acetic acid esters, N-(2, Hydroxyethyl)-2-pyrrolidone, 1-Laurylazacycloheptane, 2-(1-Nonyl)-1,3-dioxolane, 1-N-Octylazacyclopentan-2-one, N-(1-Oxododecyl)-hexahydro-1H-azepine, N-(1, Oxododecyl)-morpholines, 1-Oxohydrocarbyl-substituted azacyclohexanes, N-(1-Oxotetradecyl)-hexahydro-2-oxo-1H-azepine, N-(1 Thiododecyl)-morpholines.

Solvents and related compounds: These molecules are solubility enhancers. Some of them also extract lipids, thereby increasing skin permeability. Examples of solvents include Acetamide and derivatives, Acetone, n-Alkanes (chain length between 7 and 16), Alkanols, diols, short-chain fatty acids, Cyclohexyl-1,1-dimethylethanol, Dimethyl acetamide, Dimethyl formamide, Ethanol, Ethanol/d-limonene combination, 2-Ethyl-1,3-hexanediol, Ethoxydiglycol (transcutol), Glycerol, Glycols, Lauryl chloride, Limonene, N-Methylformamide, 2-Phenylethanol, 3-Phenyl-1-propanol, 3-Phenyl-2-propen-1-ol, Polyethylene glycol, Polyoxyethylene sorbitan monoesters, Polypropylene glycol 425, Primary alcohols (tridecanol), Procter & Gamble system: small polar solvent (1,2-propane diol, butanediol, C3-6 triols or their mixtures and a polar lipid compound selected form C16 or C18 monounsaturated alcohol, C16 or C18 branched saturated alcohol and their mixtures), Span 20, Squalene, Triacetin, Trichloroethanol, Trifluoroethanol, Trimethylene glycol, Xylene, DMSO and related compounds.

Fatty alcohols, fatty acids, fatty esters, and related structures: These molecules are classic bilayer fluidizers. These correspond to one of the most investigated class of enhancers. Examples of these enhancers include Aliphatic alcohols, Decanol, Lauryl alcohol (dodecanol), Linolenyl alcohol, Nerolidol, 1-Nonanol, n-Octanol, Oleyl alcohol, Butyl acetate, Cetyl lactate, Decyl N,N-dimethylamino acetate, Decyl N,N-dimethylamino isopropionate, Diethyleneglycol oleate, Diethyl sebacate, Diethyl succinate, Diisopropyl sebacate, Dodecyl N,N-dimethylamino acetate Dodecyl (N,N-dimethylamino)-butyrate, Dodecyl N,N-dimethylamino isopropionate, Dodecyl 2-(dimethylamino)propionate, EO-5-oleyl ester, Ethyl acetate, Ethylaceto acetate, Ethyl propionate, Glycerol monoethers, Glycerol monolaurate, Glycerol monooleate, Glycerol monolinoleate, Isopropyl isostearate, Isopropyl linoleate, Isopropyl myristate, Isopropyl myristate/fatty acid monoglyceride combination, Isopropyl myristate/ethanol/L-lactic acid (87:10:3) combination, Isopropyl palmitate, Methyl

acetate, Methyl caprate, Methyl laurate, Methyl propionate, Methyl valerate, 1-Monocaproyl glycerol, Monoglycerides (medium chain length), Nicotinic esters (benzyl), Octyl acetate, Octyl N,N-dimethylamino acetate, Oleyl oleate, n-Pentyl N-acetylprolinate, Propylene glycol monolaurate, Sorbitan dilaurate, Sorbitan dioleate, Sorbitan monolaurate, Sorbitan monooleates, Sorbitan trilaurate, Sorbitan trioleate, Sucrose coconut fatty ester mixtures, Sucrose monolaurate, Sucrose monooleate, Tetradecyl N,N-dimethylamino acetate, Alkanoic acids, Capric acid, Diacid, Ethyloctadecanoic acid, Hexanoic acid, Lactic acid, Lauric acid, Linoelaidic acid, Linoleic acid, Linolenic acid, Neodecanoic acid, Oleic acid, Palmitic acid, Pelargonic acid, Propionic acid, Vaccenic acid, a-Monoglyceryl ether, EO-2-oleyl ether, EO-5-oleyl ether, EO-l0-oleyl ether, Ether derivatives of polyglycerols and alcohols (1-O-dodecyl-3-O-methyl-2-0-(29, 39-dihydroxypropyl)glycerol), L-α-amino-acids, Lecithin, Phospholipids, Saponin/phospholipids, Sodium deoxycholate, Sodium taurocholate, Sodium tauroglycocholate.

Others: Aliphatic thiols, Alkyl N,N-dialkyl-substituted amino acetates, Anise oil, Anticholinergic agent pretreatment, Ascaridole, Biphasic group derivatives, Bisabolol, Cardamom oil, 1-Carvone, Chenopodium (70% ascaridole), Chenopodium oil, 1,8 Cineole (eucalyptol), Cod liver oil (fatty acid extract), 4-Decyloxazolidin-2-one, Dicyclohexylmethylamine oxide, Diethyl hexadecylphosphonate, Diethyl hexadecylphosphoramidate, N,N-Dimethyl dodecylamine-N-oxide, 4, 4-Dimethyl-2-undecyl-2-oxazoline, N-Dodecanoyl-L-amino acid methyl esters, 1,3-Dioxacycloalkanes, (SEPAs), Dithiothreitol, Eucalyptol (cineole), Eucalyptus oil, Eugenol, Herbal extracts, Lactam N-acetic acid esters, N-Hydroxyethalaceamide, 2-Hydroxy-3-oleoyloxy-1-pyroglutamyloxypropane, Menthol, Menthone, Morpholine derivatives, N-Oxide, Nerolidol, Octyl-β-D-(thio)glucopyranosides, Oxazolidinones, Piperazine derivatives, Polar lipids, Polydimethylsiloxanes, Poly [2-(methylsulfinyl)ethyl acrylate], Polyrotaxanes, Polyvinylbenzyldimethylalkylammonium chloride, Poly(N-vinyl-N-methyl acetamide), Prodrugs, Saline (skin hydration), Sodium pyroglutaminate, Terpenes and azacyclo ring compounds, Vitamin E (α-tocopherol), Ylang-ylang oil, N-Cyclohexyl-2-pyrrolidone, 1-Butyl-3-dodecyl-2-pyrrolidone, 1,3-Dimethyl-2-imidazolikinone, 1,5 Dimethyl-2-pyrrolidone, 4,4-Dimethyl-2-undecyl-2-oxazoline, 1-Ethyl-2-pyrrolidone, 1-Hexyl-4-methyloxycarbonyl-2-pyrrolidone, 1-Hexyl-2-pyrrolidone, 1-(2 Hydroxyethyl)pyrrolidinone, 3-Hydroxy-N-methyl-2-pyrrolidinone, 1-Isopropyl-2-undecyl-2-imidazoline, 1-Lauryl-4-methyloxycarbonyl-2-pyrrolidone, N-Methyl-2-pyrrolidone,Poly(N-vinylpyrrolidone), Pyroglutamic acid esters, Acid phosphatase, Calonase, Orgelase, Papain, Phospholipase A-2, Phospholipase C, Triacylglycerol hydrolase.

"*pharmaceutical*" or, used interchangeably, "*drug*" means any substance or compound that has a therapeutic, disease preventive, diagnostic, or prophylactic effect when administered to an animal or a human. The term pharmaceutical includes prescription drugs and over the counter drugs. Pharmaceuticals suitable for use in the invention include all those known or to be developed. Examples of suitable pharmaceuticals include, but are not limited to, cardiovascular pharmaceuticals, such as amlodipine besylate, losartan potassium, irbesartan, diltiazem hydrochloride, clopidogrel bisulfate, digoxin, abciximab, furosemide, amiodarone hydrochloride, beraprost, tocopheryl nicotinate; antiinfective components, such as amoxicillin,

clavulanate potassium, azithromycin, itraconazole, acyclovir, fluconazole, terbinafine hydrochloride, erythromycin ethylsuccinate, and acetyl sulfisoxazole; psychotherapeutic components, such as sertraline hydrochloride, venlafaxine, bupropion hydrochloride, olanzapine, buspirone hydrochloride, alprazolam, methylphenidate hydrochloride, fluvoxamine maleate, and ergoloid mesylates; gastrointestinal products, such as lansoprazole, ranitidine hydrochloride, famotidine, ondansetron hydrochloride, granisetron hydrochloride, sulfasalazine, and infliximab; respiratory therapies, such as loratadine, fexofenadine hydrochloride, cetirizine hydrochloride, fluticasone propionate, salmeterol xinafoate, and budesonide; cholesterol reducers, such as atorvastatin calcium, lovastatin, bezafibrate, ciprofibrate, and gemfibrozil; cancer and cancer-related therapies, such, as paclitaxel, carboplatin, tamoxifen citrate, docetaxel, epirubicin hydrochloride, leuprolide acetate, bicalutamide, goserelin acetate implant, irinotecan hydrochloride, gemcitabine hydrochloride, and sargramostim; blood modifiers, such as epoetin alfa, enoxaparin sodium, and antihemophilic factor; antiarthritic components, such as celecoxib, nabumetone, misoprostol, and rofecoxib; AIDS and AIDS-related pharmaceuticals, such as lamivudine, indinavir sulfate, stavudine, and lamivudine; diabetes and diabetes-related therapies, such as metformin hydrochloride, troglitazone, and acarbose; biologicals, such as hepatitis B vaccine, and hepatitis A vaccine; hormones, such as estradiol, mycophenolate mofetil, and methylprednisolone; analgesics, such as tramadol hydrochloride, fentanyl, metamizole, ketoprofen, morphine sulfate, lysine acetylsalicylate, ketorolac tromethamine, morphine, loxoprofen sodium, and ibuprofen; dermatological products, such as isotretinoin and clindamycin phosphate; anesthetics, such as propofol, midazolam hydrochloride, and lidocaine hydrochloride; migraine therapies, such as sumatriptan succinate, zolmitriptan, and rizatriptan benzoate; sedatives and hypnotics, such as zolpidem, zolpidem tartrate, triazolam, and hycosine butylbromide; imaging components, such as iohexol, technetium, TC99M, sestamibi, iomeprol, gadodiamide, ioversol, and iopromide; and diagnostic and contrast components, such as alsactide, americium, betazole, histamine, mannitol, metyrapone, petagastrin, phentolamine, radioactive B_{12}, gadodiamide, gadopentetic acid, gadoteridol, and perflubron. Still other examples of suitable pharmaceuticals are listed in 2000 *MedAd News* 19:56-60 and *The Physicians Desk Reference, 53rd. Edition*, pages 792-796, Medical Economics Company (1999), both of which are incorporated herein by reference.

Examples of suitable veterinary pharmaceuticals include, but are not limited to, vaccines, antibiotics, growth enhancing components, and dewormers. Other examples of suitable veterinary pharmaceuticals are listed in *The Merck Veterinary Manual, 8th Edition*, Merck and Co., Inc., Rahway, NJ, 1998; (1997); *The Kirk-Othmer Encyclopedia of Chemical Technology, Volume 24* Kirk-Othmer (4th Edition at page 826); and *Veterinary Drugs* by A.L. Shore and R.J. Magee, American Cyanamid Co. in *The Encyclopedia of Chemical Technology* 2nd. Edition, Vol. 21, each of which is incorporated herein by reference

"*reservoir medium*" refers to a liquid, solution, gel, or sponge that is chemically compatible with the components in a sample and the tissue being used in an apparatus or method of the present invention. In one embodiment of the present invention, the reservoir medium comprises part of the specimen taken to measure or

analyze the transfer, flux, or diffusion of a component across a tissue barrier. Preferably, the reservoir medium is a liquid or solution.

"*sample*" means a mixture of a plurality of active components and a plurality of inactive components. Preferably a sample comprises two or more additional components, more preferably, three or more additional components. In general, a sample will comprise one active component but can comprise multiple active components. Samples can be liquid source or solid source samples, which include samples in the form of solids, semi-solids, films, liquids, solutions, gels, foams, pastes, ointments, triturates, suspensions, or emulsions.

"*solid form*" means that the sample containing the component or components being measured or analyzed is in the form of a solid or semi-solid, which includes, without limitation, triturates, gels, films, foams, pastes, ointments, adhesives, high viscoelastic liquids, high viscoelastic liquids having solid particulates dispersed therein, and transdermal patches.

"*solvent*" means a fluid in which a component such as an active component, carrier, or adhesive will dissolve. Solvents are selected based on the solubility of the material to be dissolved, chemical compatibility, biocompatibility and other factors. Aqueous solvents can be used to make matrices formed of water soluble polymers. Organic solvents will typically be used to dissolve hydrophobic and some hydrophilic polymers. Preferred organic solvents are volatile or have a relatively low boiling point or can be removed under vacuum and which are acceptable for administration to humans in trace amounts, such as methylene chloride. Other solvents, such as ethyl acetate, ethanol, methanol, dimethyl formamide (DMF), acetone, acetonitrile, tetrahydrofuran (THF), acetic acid, dimethyl sulfoxide (DMSO) and chloroform, and combinations thereof, also may be utilized. Preferred solvents are those rated as class 3 residual solvents by the Food and Drug Administration, as published in the Federal Register vol. 62, number 85, pp. 24301-24309 (May 1997) which is incorporated herein by reference. Solvents for drugs will typically be distilled water, buffered saline, Lactated Ringer's or some other pharmaceutically acceptable carrier.

A. Overall description of method

A schematic of the workflow for one class of experiments is shown in FIG. 1. A donor array plate is provided, equipped with an array of isolated electrodes, one for each donor well. For the number of distinct wells in the provided donor plate, an experimental design is completed to determine the composition of samples to be loaded into each donor well in the donor array. This design will typically include a suitable number of replicates, blanks and standards. Software such as described by Strehlau et al. and by Newsam et al. [14,15], both of which references are incorporated herein by reference, may conveniently be used to help in the experimental design choice. According to this experimental design the components for each of the donor well compositions are introduced by a manual pipette or similar manual device or, preferably, by using a liquid- and/or solid- and/or soft solid- handling robotic device.

The contents of the wells in the array are mixed, such as by using a vortexing orbital shaker. The lamina is provided, typically, as described further below, a suitable piece of porcine skin, and placed on top of the donor array plate. At this stage, there is no

contact between the contents of any of the donor wells and the lamina. A receptor array plate is then mounted on top of the lamina and the receptor wells are filled, typically with PBS. A device such as illustrated in FIGS. 5-7 is used to ensure complete filling of all receptor wells and elimination of all bubbles. The assembly is clamped firmly together, this clamping ensuring that the lamina effectively seals all of the donor wells and all of the receptor wells. To commence the permeation experiments, the assembly is inverted, the leak valves on the donor wells are opened and the assembly is placed on an orbital shaker to ensure that the air pockets in the donor wells are eliminated as the air passes up to what are now the tops of the donor wells. Polling of the electrical response of the lamina in the vicinity of each donor/acceptor well is commenced immediately after inversion of the assembly, and this polling is continued throughout the experiment duration, typically 6 hours, although longer runs to 24 or 28 hours may also be performed.

In addition to the accumulation of the electrical response of the lamina at the position of each donor well, direct measurement of the concentration of a test reagent in the donor and/or receptor wells may be performed. This is achieved by abstracting small liquid samples from donor and receptor wells. The abstraction from the donor wells can, in the embodiment currently being described, be made by using a manual pipette or, preferably, an automated fluid handler, as the donor wells are uppermost and open. The abstraction of small amounts of donor fluid does not affect the concentration of the test reagent in the donor well, nor significantly perturb the experiment. Abstraction of small amounts of receptor fluid from the closed and fully-filled receptor wells is achieved using the device shown in FIG. 8.

A further experimentation possibility is that the concentration of a test reagent layer by layer through the stratum corneum is measured. According to the present invention, this is conveniently achieved in an array format using the device shown in FIG. 9. At the end of the experiment duration, the contents of all donor wells are removed by aspiration and both the wells and the lamina surface exposed in each well is gently washed. The clamp and the receptor plate are removed and the bottom surface of the lamina is gently washed. The apparatus is inverted and the donor array plate removed. The lamina is then placed on the base of the stripping-roller device (FIG. 9) and successive layers are removed from an area on the lamina encompassing the positions of several donor wells, typically of all donor wells. Successive adhesive sheets then contain successive layers through the lamina. Each sheet is then analyzed using an array reader. Depending on the nature of the test reagent, the analysis may be performed with a UV-vis spectrophotometer, a fluorescence spectrometer, or a scintillation counter, in each case arranged for plate reading.

Additional types of experiments that are possible with the methods and devices of the present invention are described below.

B. Description of apparatus

Overall layout: The array format used with the present invention may be any one of many described previously in the literature. For example, a 24, 36, 48, or 96 well plate format such as available from Millipore, Bedford, MA (www.millipore.com) might be chosen. There are advantages to employing a commonly-used format, such as the 96-,

384- or 1536-well plate formats, as these, particularly the 8x12 96-well microtitre plate format, are compatible with a broad range of automation and software solutions. There are also advantages to using a linear array format, as the individual wells can be accessed simply in a linear fashion. A close-to-linear format as disclosed in US Patent 5,490,415 [6] may have intermediate advantages. The benefits of the present invention can be realized using any of these array formats, and the choice as to which array format to employ in a particular case is generally made based on factors such as (i) the format(s) in which suitable lamina are available, (ii) compatibility with established automation and software solutions, and (iii) preferred volume and diameter of donor and receptor wells (guided by cross-talk considerations; engineering issues such as with bubble elimination, agitation, abstraction, lamina uniformity, reagent availability etc.). Associated with the chosen array format, the sample can comprise less than about 100 milligrams of the active component, preferably, less than about 1 milligram, more preferably, less than about 100 micrograms, and even more preferably, less than 100 nanograms. Preferably, the sample in a given well has a total volume of about 1-200 µl, more preferably about 5-150 µl, and most preferably about 10-100 µl.

Central plates: While the general format of the donor well array may be any one of many formats described previously in the literature, a unique design of donor and acceptor array plates minimizes 'bleeding' or cross-talk between adjacent wells. This unique design also accommodates the *in situ* cutting of the lamina into strips or discrete pieces (FIG. 3B).

Fast assembly: Previous implementations of array formats for testing the permeation of agents through a membrane have used simple screws as a means of connecting donor and receptor well plates and applying pressure to the interleaving membrane. This closure mechanism has major disadvantages in (i) reproducibility of the pressure being applied, (ii) uniformity of the pressure across the membrane surface, and (iii) time and convenience cost associated with applying and removing the bolts or screws. A substantial improvement is provided in the present invention by using a unique clamping mechanism (FIG. 4) that is rapid and provides an adjustable and accurately reproducible clamping pressure.

Air pocket-free and bubble-free well-filling: A prime benefit of the present invention is that it provides for the full filling of wells with avoidance of bubbles or air pockets.

A method for filling wells that ostensibly avoids the occurrence of air pockets or bubbles between a sample and a tissue is described in WO 02/06518 A1 [8]. A feed canula, having a sample feed source and an air evacuation space, punctures a rubber septum which covers one side of the donor well. By placing the tip of the canula on the tissue it was anticipated that the air in the well would be forced out of the well into the air evacuation space, eliminating any air pockets adjacent to the tissue. The tip of the canula, further, is progressively retracted toward the septum as filling proceeds. However, this method requires contact of the sharp tip of the canula with the tissue, causing damage to the barrier layers on the top of the tissue. Further, without sophisticated methods it is difficult both to determine to precisely what depth the canula must be inserted (leading to the possibility of severe tissue barrier damage), and the extent to which well filling has progressed (making concerted retraction of the canula difficult to control). Further, this 'from near the bottom introduction' method is not effective in practice at eliminating bubbles, particularly for viscous samples. Additionally,

this approach is not claimed to be useful in achieving complete filling of a well compartment.

The present invention provides methods and devices for the air pocket- and bubble-free loading of a well or of wells in an array (FIG. 5, FIG. 6, FIG. 7).

Device rotation: The methods of achieving air pocket- and bubble-free well loading enable permeation and other skin impact assessment experiments to be performed in novel ways.

Firstly, the donor plate – lamina – receptor plate assembly can be prepared in an inverse fashion, namely receptor plate – lamina – donor plate, as was described in the General Description of the Method. As it is essential to maintain contact between the fluids in each of the receptor wells and the lamina throughout a given experiment, without a method and device for achieving complete well filling, the array format will always present concerns and, given the greater importance of ensuring donor sample contact with the membrane during the experiment, experiments had to be effected in the prior art by loading the donor wells in a manner that involved immediate contact with the membrane. To achieve air pocket- and bubble-free, complete well loading, the device illustrated in FIG 6 or FIG. 7I is used.

With this method and device, an array of receptor wells that is sealed on one surface by a lamina can be completely filled, ensuring fluid contact with the lamina. These same devices are again applied when small amounts of fluid are to be abstracted from one or more receptor wells, to ensure avoidance of air pocket or bubble formation. The opposite donor wells can then be loaded prior to contact with the lamina, as described in the General Description of the Method.

With these methods and devices, the assembled apparatus can be orientated in any fashion without disturbing the contact of the donor or receptor samples with the lamina. Thus permeation experiments may be performed with the receptor well array uppermost, with the donor well array uppermost, with donor and receptor arrays vertical, or at any other angle. Additionally, the apparatus can be shaken or agitated without affecting the sample contact with the lamina, greatly extending the utility of arrays for screening experiments. Samples that contain components that would otherwise cream or settle can be used; exfoliation experiments with an *in situ* agitation may also be performed.

Receptor well sampling: The present method and devices permit specimens to be withdrawn from one or more donor or receptor wells at an appropriate time or after each of various time intervals. Such abstractions are used to measure directly the transfer or flux of components, such as active components, in samples across the lamina. Generally, the volume(s) of specimens withdrawn are such that the reduction in quantity of donor or receptor medium has no influence on the overall experiment; if multiple specimens are taken, however, after a volume of specimen is removed from a donor well, a volume of donor well sample may be added to the same donor well for replenishment, although the composition of the sample to be added must match that present in the donor well at the time of the addition. Abstraction from the donor or receptor wells can be made by using a manual pipette or, preferably, an automated fluid handler, when the donor wells are uppermost and open, or sealed with a septum. Abstraction of small amounts of donor or receptor fluid from inverted, closed and fully-filled receptor wells is achieved using the device shown in FIG. 8.

Detection of the presence or absence, or measurement of the concentration of active components or test reagents in the abstracted fluids may be made by any one of several methods. High performance liquid chromatography ("HPLC"), or any one of several known spectroscopic techniques can be used. Suitable measurement techniques include, but are not limited to include spectroscopy, infrared spectroscopy, near infrared spectroscopy, Raman spectroscopy, NMR, radioactivity monitoring coupled with the use of a radiolabelled active component or test reagent.

Electrical response measurements: Measurements of skin conductivity and permittivity have a long history, providing a reasonable data base against which new measurements can be compared and validated. Additionally, Karande et al. [9,10] have disclosed the use of skin conductivity as a proxy measure of the permeability of the stratum corneum.

The present invention provides novel means of making conductivity measurements, on lamina regions or pieces, in high throughput and discloses the use of electrical response measurements in high throughput, in an array format.

Firstly, an array of donor wells is provided with an integral array of electrodes, one for each donor well (FIG. 13). The electrodes are arranged to be electrically insulated one from another, yet each makes good electrical contact with the fluid contents of the donor well (FIG. 13).

Secondly, the electrode array is arranged for simple, fast and robust establishment of electrical connections with the measurement circuitry (FIG. 14) as the experiment is prepared.

Thirdly, the electrical circuitry is organized such that electrical response measurements can be made in a bridge array (FIG. 14B).

Fourthly, in contrast to the prior art devices which are suitable only for relatively non-viscous and highly-conductive aqueous solutions, the present devices can accommodate measurements of electrical response of a lamina in contact with samples that can be solids, soft materials, or poorly electrically conductive media. Such measurements are made possible by the application of a gel electrode to the area of lamina exposed in each well.

Additional permeation rate measurement method: An alternative means of measuring the rate of permeation of a compound through a lamina is enabled by the device disclosed here, that allows for essentially simultaneous contact of the formulations in a set of donor wells with the lamina. The relative permeation rates of compounds in the set of donor wells can be determined by initiating commencement of contact, and hence penetration, at essentially the same time and then monitoring, on the receptor side of the lamina opposite each donor well, the emergence of the corresponding compound as a function of time after this initial contact. For example, into the set of donor wells are placed a given concentration of a hydrophilic drug, together with a combination of permeation enhancers ("CPEs"). Each donor well receives a combination of CPEs according to a design, based on a useful sampling of the CPE combination space, together with a suitable number of replicates, references and blanks. A replicate is a composition in a donor well identical to that in another donor well, with the number and positioning in the donor well array of the replicates of a given composition chosen so as to provide the desired checks and measurement statistics. A blank is a donor well that is left unfilled, or which is filled with a composition that is missing the compound to be

monitored. A reference is a composition for which permeation has been separately measured and which, therefore, can be used as a reference. The contents of all donor wells are simultaneously brought into contact with the lamina and from that experiment start time, t_0, the receptor well side of the lamina is monitoring by a method or device that is indicative of the presence of the permeating compound. This device might use any known method, such as detection of color by UV-vis spectroscopy, detection of fluorescence, binding to an agent to generate color or fluorescence etc. From the time taken for the analyte to permeate through the lamina and be detected on the receptor well side, an approximate indication of the permeation rate can be deduced.

Application and manipulation of complex samples: A device suitable for the application of a soft material as a sample or as a sample component is illustrated in FIG. 11. The soft material is loaded into a syringe, any manner of which might be used; the syringe may be manually or, preferably, automatically activated. When the plunger is depressed, soft material is expelled from the syringe barrel, into a large-bore tube with a flexible end section. Once the tube is filled from the syringe, the collapsible region of the tube is caused to collapse in a progressive fashion by introduction of gas or, preferably, fluid into the region between the flexible tube section and a rigid outer barrel. The soft material in the collapsible section is then expelled from the tube, into the well, completing the controlled delivery.

An additional complication with viscous samples is the potential for the establishment of air pockets, particularly at the sample – lamina interface. Such air pockets can be eliminated, according to the present invention, by the application of ultrasonic agitation to an individual well or, preferably to the entire array of samples (FIG. 12). Suitable ultrasonication baths are available from many suppliers such as Nickel–Electro Limited of Weston Super Mare, UK (www.martex.co.uk/blwa/nickel/ultra.htm), and Branson Ultrasonics Corporation of Danbury, CT (www.bransonultrasonics.com).

This same device is also beneficially applied to disrupting the microstructure of soft material samples. Many skin lotions are emulsions or reverse emulsions and the process of rubbing the lotion onto the skin has the effect of disrupting the droplet microstructure or 'breaking' the emulsion, liberating the emulsion droplet contents. To perform effective screening of such skin lotion formulations, therefore, it may be necessary to break the emulsion before skin penetration or absorption screens are applied, for which the present device can be usefully applied (FIG. 12).

Ultrasonic sampling: This present invention also discloses a method of characterizing the disruption of lipid structures in the stratum corneum by application of ultrasonic spectroscopy. Following exposure of the lamina to a sample for an appropriate time, a plug of the lamina is removed and placed in an ultrasonic spectrometer, such as is available from Ultrasonic Scientific of Piscataway, NJ (www.ultrasonic-scientific.com). Ultrasonic waves propagate through opaque biological tissues and recent instrumentation developments have improved the resolution and the limitations on sample size to an extent that useful information on stratum corneum structure can be derived from ultrasonic measurements.

Layer-by-layer analysis: Another aspect of the present invention is the ability to perform layer-by-layer analysis of the lamina in an automated fashion. The device used to abstract successive layers from the lamina is illustrated in FIG. 9. The lamina is placed on a base which is driven forward, underneath a roller, by an electrical motor. The roller

is equipped with a device that ensures that the roller is applied to the lamina with a uniform pressure, which can be adjusted by the user. The exterior surface of the roller is provided with concentric pieces of one-sided sticky tape, of a length more than that of the lamina. Various roller diameters may be used, to accommodate various lamina sizes. The sticky side of the tape is applied to the lamina, as it is uniformly passed beneath the roller, causing rotation of the roller and ensuring that each area on the lamina is contacted by a different area of sticky tape. Reference marks can be applied to the tape to ensure that a position on the tape can be referenced back to the corresponding position on the lamina surface. Alternatively, a reference material or materials can be applied to the lamina before stripping to provide suitable reference marks on the tape. Once the lamina has passed completely underneath the roller, the roller is lifted and the tape piece is removed, exposing a next clean piece of tape, sticky side towards the outside of the roller. The base and lamina are repositioned back at the start of travel, the roller is replaced, and the process is repeated the desired number of times. The set of tape pieces applied to the lamina then contains, in sequence, the set of layer abstractions from the lamina. The individual tape pieces may then be analyzed in any established way, such as by direct chemical analysis, spectroscopically or otherwise. When radiolabelled components are used in a sample, the tape strips may be counted in a suitable scintillation counter.

C. Description of application and measurement types

'One shot' measurement of permeation and permeation enhancement: Measurements are performed generally as outlined in the Overall Description of Method above. A donor plate with a standard array geometry, 96 donor wells in an 8 by 12 array, is provided. In the present example, rather than standard microtitre plate well size of 200µl volume, 6.5mm well diameter, a well diameter of 1.5mm is chosen. According to the experimental design, ingredients are provided into each of the donor wells by a commercial fluid dispensing robot. The liquid volume is chosen so that each of the wells is about 75% full of sample, with no superfluous sample on the top donor plate surface between wells. The donor plate is placed on a vortexing orbital shaker to intimately mix the contents in each of the 96 donor wells, making sure that no sample is shaken out into the top space of the donor well plate between the wells.

A lamina in the form of porcine skin is placed on top of the donor well array with the stratum corneum facing the donor plate. The fact that none of the donor wells is full ensures that there is initially no contact between the sample in each donor well and the skin; the permeation experiment has not yet commenced. Porcine skin can be harvested from Yorkshire pigs and stored at –70°C immediately after procurement until the time of experiments. The skin is thawed at room temperature prior to each experiment. A receptor plate, of suitable design (either an 8 by 12 array of separate wells that is the mirror image of the donor well layout (FIG. 2A), or a common bath, with a suitable arrangement of posts to provide mechanical support (FIG. 2B)) is placed on top of the skin. The receptor wells are filled with PBS to keep the skin hydrated over the entire duration of the experiment (24 hrs). A clamp is then applied to the donor plate–skin–receptor plate assembly (FIG. 4) to keep it clamped closed and ensure sealing of each donor and receptor well by the skin.

The assembly is inverted, and the assembly is again agitated on an orbital shaker to ensure that any bubbles or air pockets at the lamina sample interface are dislodged and

rise to the top of the donor wells, on the opposite end of the wells from the skin. Contact of donor well samples with the skin is maintained for the experiment duration (24 h), then the clamp is removed. The fluid dispensing robot is used to aspirate fluid from each of the donor wells, loading the aspirate into septum-sealed vials which are provided for analysis by HPLC or similar. The donor plate and skin are then removed and the fluid dispensing robot is, similarly, used to aspirate material from each of the receptor wells into septum-sealed vials which are also provided for HPLC or other analysis. These measurements yield direct data on the amount of active component, and/or of other components in the samples, that have permeated through the skin in the experiment time period. From the difference in donor and receptor well concentrations, knowing the amounts of fluids introduced at the outset, the amount of the various constituents absorbed by the skin can also be deduced.

Near-continuous measurement of electrical response: When electrical response measurements will be applied as a screening vehicle, the procedure is similar, but a donor array plate that is equipped with an array of electrodes is used. A counter electrode is inserted into the epidermis of each of the lamina pieces (or a single counter electrode is applied should the skin not be cut into strips). After filling of donor and receptor wells, with mixing if required, the necessary electrical connections are made (in the case of ring or other electrode included as integral parts of the donor plate). The clamped assembly is then inverted so that the solutions in all 96 donor wells come, simultaneously, in contact with the skin (FIG. 3) and the assembly is again agitated on an orbital shaker to ensure that any bubbles or air pockets at the lamina sample interface are dislodged and rise to the top of the donor wells. This is the time of experiment commencement, t_0.

The analog switches are set such that connections are made to the first set of 12 ring electrodes in the first row of wells in the microtitre plate. All 12 measurements could be made simultaneously, but, in practice, it is more convenient to use a single set of control electronics and poll the 12 wells in turn, piping the data appropriately to the data management system.

In turn the counter electrode (inserted into the epidermis of the strip) and the electrode mounted into the defined donor well are connected to the drive circuitry. A 10 or 20 μsec square voltage pulse is applied and the control software accumulates the data as a function of time, Fourier transforms the response, correlates the response parameters with age, thickness, permeability, and then transfers the raw, filtered, transformed, and interpreted data, according to user preferences, to the data storage system.

The circuitry is then switched to apply the same measurement to the next cell in the set of 12, pinging each and then measuring the response. Once the set of 12 cells have been polled, the analog switch is then switched to the next set of 12 donor wells and the measurements are repeated. The measurement time per well can be as short as millisecs, so a row of 12 is done is completed almost simultaneously. This polling is repeated for the time-duration of the measurement, with sampling and/or data storage being made according the user-set preferences.

Permeabilities using a radiolabelled component: Passive permeabilities of an active component through a lamina can also be measured using trace quantities of a radiolabelled active component. According to known methods, radiolabelled active components are rotary evaporated in order to remove any solvent in which they are

shipped and any tritium which had reverse exchanged into it. The radiolabelled active component is then redissolved in a suitable solvent and combined into, or redissolved directly within each of various sample compositions, including enhancers, carriers, additives, and/or other excipients, to a typical concentration of 1 μCi/ml. The mixtures may be prepared separately and added to the donor wells, or two or more of the constituents may be separately added to the donor wells and then mixed within the donor wells, as described above. The receptor wells are preferably loaded with, for example, pH 7.4 phosphate buffer saline (PBS, phosphate concentration = 0.01 M, NaCl concentration = 0. 137 M; available from Sigma-Aldrich Inc., www.sigmaaldrich.com). The concentrations of the radiolabelled active component in the donor and receptor wells are measured using a scintillation counter (e.g., TopCount NXT available from PerkinElmer Life and Analytical Sciences, Inc. of Boston, MA (las.perkinelmer.com)). As described above, the experiment design for the compositions of the different samples in the donor array will typically entail a number of replicates, one or more blanks and, possibly, a calibrating standard or standards.

The permeability values can be calculated under steady-state conditions from the relationship $P = (dN_r/dt)/(AC_a)$ where A is the surface area of the lamina accessible to a sample, C_a is the concentration of the active component in the sample, and N_r is the cumulative amount of active component which has permeated into the receptor reservoir. There is significant inter-species and inter-individual variability in skin permeability; for example, an inter-individual variation in human skin of 40% is reported by Williams, et al. [16]. The passive permeability enhancement, EP, is calculated relative to the passive permeability from PBS according to Eq. (1).

$$E_P = \frac{P_{(enhancer)}}{P_{(PBS)}} \qquad (1)$$

where $P_{(enhancer)}$ is the permeability of the active component in the presence of the other sample constituents, and $P_{(PBS)}$ is the permeability of the active component from PBS. The fluxes from saturated solutions, J^{sat}, are calculated from $J^{sat} = P\,C^{sat}$, where C^{sat} is the drug solubility in the sample formulation. Flux enhancements, E_J, are calculated using Eq. (2),

$$E_J = \frac{J^{sat}{}_{(enhancer)}}{J^{sat}{}_{(PBS)}} \qquad (2)$$

where $J^{sat}{}_{(enhancer)}$ and $J^{sat}{}_{(PBS)}$ are the fluxes of active component from saturated solutions of enhancer and PBS, respectively.

Short contact time measurements: The present inventive method and devices provide a major benefit in that measurements can be commenced at short times following first contact of donor well samples with the lamina. The assembly is prepared as described in the General Description of the Method, and the electrical circuits are configured and tested. The assembly is then inverted and then agitated, to eliminate air pockets or bubbles at the donor sample – lamina interface. The electrical response measurements are commenced immediately upon inversion of the assembly, and continue through the

agitation and until the experiment is concluded. A first electrical response measurement can be made readily within 10 sec of first contact, or within 1 sec of first contact.

Measurement of uptake: A measure of absorption by the lamina of a component in a donor well may be made based on the differences in concentrations of the donor and receptor wells, relative to the initial concentrations. Taking such measurements at various contact times improves the absorption measurement.

A complementary, automated analysis is possible by using straight-through donor wells, with a removable cover. The procedure is as described above in the General Description of the Method, with the clamped assembly being inverted to commence contact by the donor well samples with the lamina. At this point the donor well cover is removed so that the donor wells can be accessed from above by a liquid handling robot. At an appropriate time for each given well, the donor sample is removed by aspiration (and typically saved in a vial for HPLC analysis); the well is then refilled with PBS. After a second time period, the amount of active that has diffused back out of the skin into the donor well PBS solution is analyzed, typically by HPLC. A set of different wells will typically be employed so as to sample both absorptive uptake and release so that the time profile of both can be ascertained.

A direct analysis is also possible by (manually) punching-out a piece of skin from the targeted donor well(s), digesting the piece of skin with NaOH and then subjecting the remains to HPLC (or other) analysis.

Uptake can be assessed directly using a radiolabelled component. Radiolabelled mannitol prepared in PBS at a concentration of 10 µCi/ml is added to all wells in a donor array plate that have been pre-loaded with a set of formulations, according to an experimental design. The donor well array is agitated to ensure complete mixing within each donor well. The skin was then incubated in contact with the array of donor well samples for 7 h. After the end of incubation period the samples are removed from each of the donor wells, the skin accessible in each donor well is rinsed gently to free any mannitol that could be sticking to the surface of the skin. The skin in each donor can then be punched and dissolved in 0.5 M Solvable™ (an aqueous based solubilizer of tissue and gel available from PerkinElmer Life and Analytical Sciences, Inc. of Boston, MA (las.perkinelmer.com) at 60°C overnight. A 500 µL sample can then be taken and the concentration of radiolabelled mannitol measured using a scintillation counter such as the Tri-Carb 2100TR available from PerkinElmer Life and Analytical Sciences, Inc. of Boston, MA (las.perkinelmer.com).

Irritation potential measurement: A benefit of the present inventive method and devices is that measurements of dermal irritation can be performed using the same apparatus as is used for measurements of permeability and absorption. Further measurements of irritation potential can be made simultaneously with other measurements, such as of permeation. Irritation measurements are made on live tissue. As an example, EpiDerm™ a skin model human-derived epidermal keratinocytes available from MatTek Corporation, Ashland, MA (www.mattek.com) is used. The procedure is generally as described above in the General Description of the Method, but an array of straight-through receptor wells is employed (FIG. 2C) and, after filling and clamping, the assembly is not inverted. Rather a device is applied to bring, simultaneously, all donor well samples into contact

with the skin. The assembly is positioned in the volume that is accessed by a fluid dispensing robot, and by a lens/CCD camera. At the time that is predetermined for each receptor well, the PBS solution in the given receptor well is removed (and can be stored in a vial for permeation analysis if desired), the receptor surface of the skin flushed with PBS, and then an appropriate amount of MTT solution is added to that well. After the preset incubation time, the supernatant MTT solution is removed, the camera is moved so as to image the well and the intensity of coloration in the accumulated image is analyzed so as to yield the activity of the epidermis by comparison with a calibration curve, and the proportion of nonviable epidermal cells is then derived. While electrical response measurements can be accumulated throughout the irritation potential tests, it is noted that the permeability of the stratum corneum in EpiDerm™ and similar synthetic tissues is typically greater then that found in natural samples.

Screening of exfoliation: The present method and devices provide for at least two ways of performing rapid screening of the loss of stratum corneum material, through exfoliation or sloughing off of clusters under the action of an emollient.

Straight-through donor wells, with a removable cover, are used. The procedure is as described above in the General Description of the Method, with the clamped device being inverted to commence contact with the skin by the donor well samples. At this point the donor well cover is removed so that the donor wells can be accessed by the liquid handling robot. The clamped device is also placed on an orbital shaker. At the defined time for the given well, a fiber-optic light source and detector are introduced into the well and the amount of sloughed–off cells or cell clusters assessed by increase in turbidity of the donor well medium. This measurement can be applied at various time intervals, or the light source and probe can be left in the donor solution and data accumulated from that one donor well as a function of time.

A destructive test is also possible. After the predetermined contact time for the given well the donor solution is aspirated and placed in a vial. A suitable amount of a solution containing a dye that will color in the presence of protein is introduced, the vial agitated and the intensity of the color recorded by a colorimeter, the intensity being related, through suitable calibratory data, to the amount of material lost from the skin surface.

General application comments: The methods and devices of the present invention have a number of beneficial applications, for example, to develop (i) optimal compositions or formulations comprising one or more active components and one or more inactive components for achieving desired characteristics for such compositions or formulations, (ii) optimal adhesive/enhancer/excipient compositions for compatibility with an active component or drug, (iii) optimal active component or drug/adhesive/enhancer/additive compositions for maximum drug flux through stratum corneum, (iv) optimal active component or drug/adhesive/enhancer/additive compositions to minimize cytotoxicity.

What is claimed is:

1. An article for assaying the interaction of ingredients in a first plurality of formulations with skin that comprises:
   (i) A second plurality of donor wells that can each be loaded without contacting the skin,
   (ii) A skin piece or a third plurality of skin pieces provided such that one side of each such donor well is sealed by a skin piece,
   (iii) A fourth plurality of receptor wells, provided such that one side of each such receptor well is sealed by a skin piece.

2. The article of Claim 1 in which each of said second plurality of donor wells is provided separately with an electrode.

3. The article of Claim 1 wherein uninterrupted contact between the fluid contents of said second plurality of donor wells and the skin, and/or the fluid contents of said fourth plurality of receptor wells and the skin is provided, irrespective of the orientation of the article.

4. The article of Claim 1 in which a plurality of said second plurality of donor wells is provided with a device for the removal of gas or liquid from a partially filled well that comprises a circular aperture in the bottom of the well, providing the entrance to an exit channel, which can be sealed by a magnetic ball.

5. The article of Claim 1 in which a plurality of said second plurality of donor wells is provided with a device for the removal of gas or liquid from a partially filled well that comprises a collapsible segment at the end of which is a one-way valve.

6. The article of Claim 1 in which said second plurality of donor wells and said fourth plurality of receptor wells are equal in number, and mirror images of one another in layout such that each receptor well receives permeate from a single donor well.

7. An article that provides for the removal of gas or liquid from a partially filled well without the introduction of bubbles that comprises a circular aperture in the bottom of the well, providing the entrance to an exit channel, which can be sealed by a magnetic ball.

8. An article that provides for the removal of gas or liquid from a partially filled well without the introduction of bubbles that comprises a collapsible segment at the end of which is a one-way valve.

9. An article for holding together a donor plate, a plurality of pieces of skin, and a receptor plate that comprises two separate sprung steel pieces in a rectangular geometry, each of which has its two long sides bowed inwardly.

10. An article for applying a removable clamp to an assembly comprising a donor plate, a plurality of pieces of skin, and a receptor plate, that comprises a base and

an arm, the arm applying force, with mechanical leverage, to two opposite points in such assembly, causing said clamp to lock closed.

11. A method of assessing the impact on skin of ingredients in a first plurality of formulations that comprises:
- (i) Providing a second plurality of formulations in separate donor wells in a planar arrangement,
- (ii) Sealing each of such donor wells with skin,
- (iii) Providing a third plurality of receptor wells,
- (iv) Providing an electromagnetic imaging device to accumulate an image of the dermal side of the skin, through the receptor wells.

12. A method of measuring in high throughput the effectiveness of each of a plurality of chemical agents as an exfoliant that comprises:
- (i) Providing a second plurality of separate donor wells in a planar arrangement, with the bottom of each well formed by a piece of skin which seals the bottom of the well,
- (ii) Providing a second plurality of formulations into separate donor wells,
- (iii) Agitating the contents of each of the donor wells,
- (iv) Measuring the amount of skin debris suspended in the donor solution.

13. The method of Claim 12 in which the amount of skin debris suspended in the donor solution is measured by light scattering.

14. The method of Claim 12 in which the amount of skin debris suspended in the donor solution is measured by introducing a dye that will stain in the presence of protein and measuring optically the intensity of coloring in the suspension.

15. A method of assessing characteristics of a skin sample that comprises the steps of:
- (i) Applying a voltage pulse between an electrode embedded in the internal skin layers and a second electrode in a conducting liquid in contact with the outermost skin surface,
- (ii) Monitoring the electrical response as a function of time after commencement of application of such voltage pulse,
- (iii) Providing the Fourier transform of such electrical response as a function of time,
- (iv) Comparing such Fourier transform with similar Fourier transformed signals from model materials or with simulation.

16. A method of measuring the permeation of compounds in a library of compositions that comprises the steps of:
- (i) Providing a first plurality of compositions into a second plurality of donor wells,
- (ii) Causing the contents of each donor well to come into contact with a lamina more or less simultaneously,
- (iii) Monitoring the opposite side of the lamina for the appearance of actives a at the regions of the lamina receptor side opposite the donor wells,

(iv) Deducing the absolute or relative permeation rates for each composition from its corresponding emergence time.

References

[1] Foldvari M, Baca-Estrada ME, He Z, Hu J, Attah-Poku S, King M: **Dermal and transdermal delivery of protein pharmaceuticals: lipid-based delivery systems for interferon alpha**. *Biotechnol. Appl. Biochem.* 1999, **30**:129–137.

[2] Leyden JJ, Rawlings AV (Ed): *Skin Moisturization* Carol Stream, IL: Allured Publishing Corp.; 2002.

[3] Ghyczy M, Vacata V: **Hydroxyacids.** In *Skin Moisturization.* Edited by Leyden JJ, Rawlings AV: Allured Publishing Corp.; 2002:323.

[4] Ussing HH, Zehran K: **Active transport of sodium as the source of electric current in the short-circuited isolated frog skin.** *Acta Physiol. Scand.* 1951, **23**:110-127.

[5] Grass GM: **Device and method for circulating fluid over a membrane.** US Patent 5,599,688, 1997.

[6] Mak VHW, Francoeur ML: **Diffusion test apparatus and method**. US Patent 5,490,415, 1996.

[7] Selick HE, Smith GA, Tolan JW: **Multi-well single-membrane permeation device and methods**. US Patent 6,043,027, 2000.

[8] Cima MJ, Chen H, Gyory JR: **System and Method for Optimizing Tissue Barrier Transfer of Compounds.** WO 02/06518 A1, 2002.

[9] Mitragotri S, Karande P: **A Combinatorial Method for Rapid Screening of Drug Delivery Formulations**. WO 02/16941 A2, 2001.

[10] Karande P, Mitragotri S: **High Throughput Screening of Transdermal Formulations.** *Pharmaceutical Research* 2002, **19**:655-660.

[11] Nilsson GE: **Measurement of water exchange through skin.** *Medical and Biological Engineering & Computing* 1977, **15**:209-218.

[12] Tagami H: **Impedance Measurement for Evaluation of Hydration State of Skin Surface.** In *Cutaneous Investigation in Health & Disease.* Edited by Lévêque J: Marcel Dekker; 1989:79.

[13] Imhof B, O'Driscoll D, Xiao P, Elliott B: **New method of Measuring Trans-epidermal Water Loss (www.eeie.sbu.ac.uk/research/photo/publications/Sensors99.PDF)**. In *SCII Conference, Cardiff, UK*: 1998.

[14] Strehlau W, Newsam JM, Demuth D, Stichert W, Brenner A, Schunk SA, Klein J: **Computer-aided optimization of substance libraries.** WO Patent 01/97152A2 (and German Patent DE 100 28 875 A1), 2001.

[15] Newsam JM, Schunk SA, Klein J: **Process for producing a multiplicity of building blocks of a library of materials".** WO Patent WO 02/43860A2 (also German Patent DE 100 59 890 A1), 2002.

[16] Williams AC, Cornwell PA, Barry BW: **On the non-Gaussian distribution of human skin permeabilities.** *Int. J. Pharmaceutics* 1992, **86**:69-77.

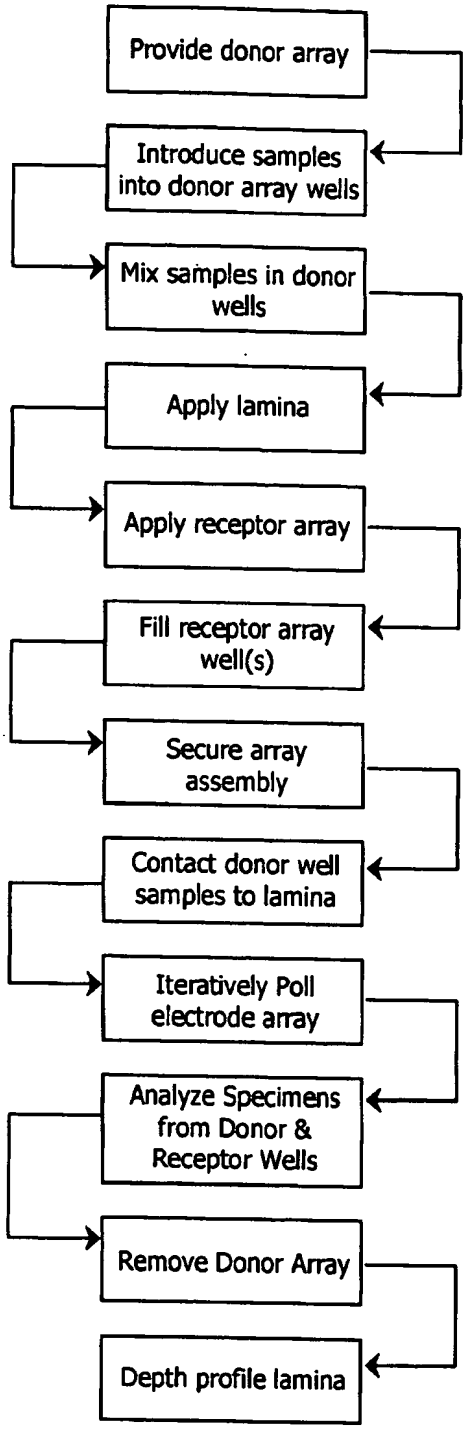

Provide donor array
Introduce samples into donor array wells
Mix samples in donor wells
Apply lamina
Apply receptor array
Fill receptor array well(s)
Secure array assembly
Contact donor well samples to lamina
Iteratively Poll electrode array
Analyze Specimens from Donor & Receptor Wells
Remove Donor Array
Depth profile lamina

FIG. 1

FIG. 2A

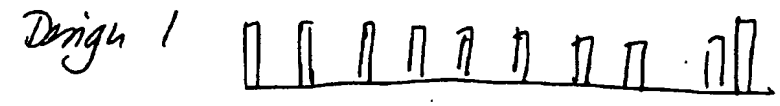


FIG. 2B

Design 2 (common post, posts for support)

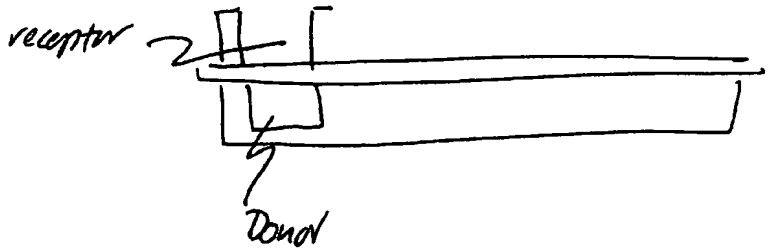


FIG. 2C

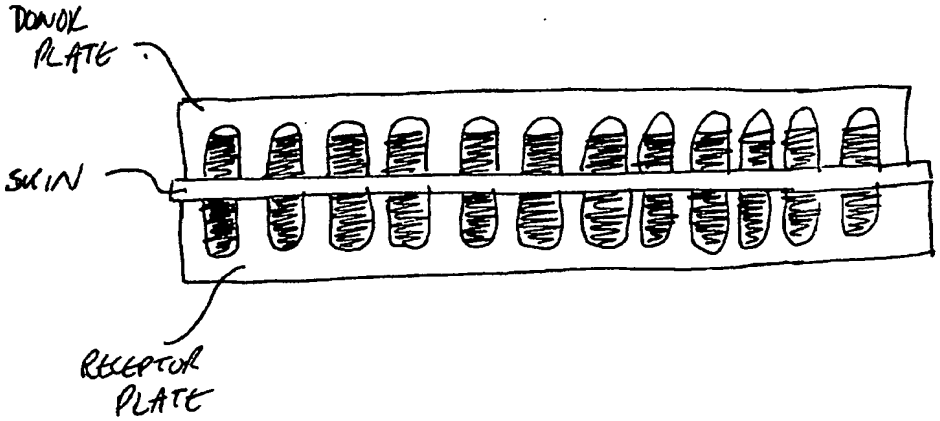


FIG. 2D

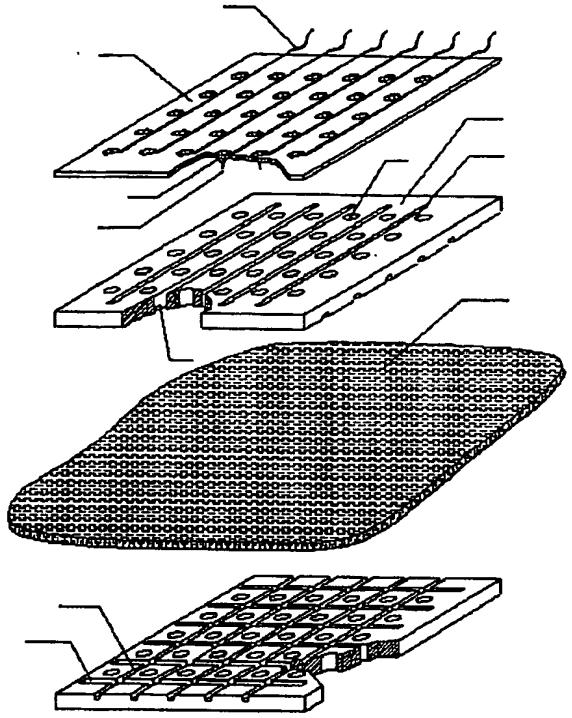


FIG. 3A

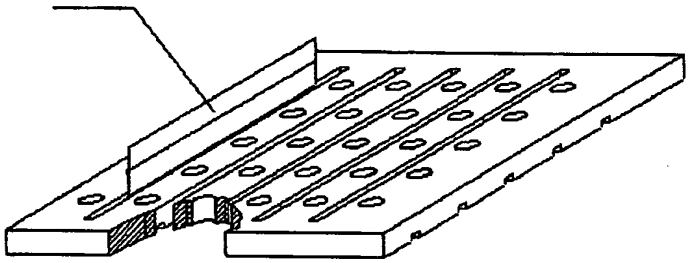
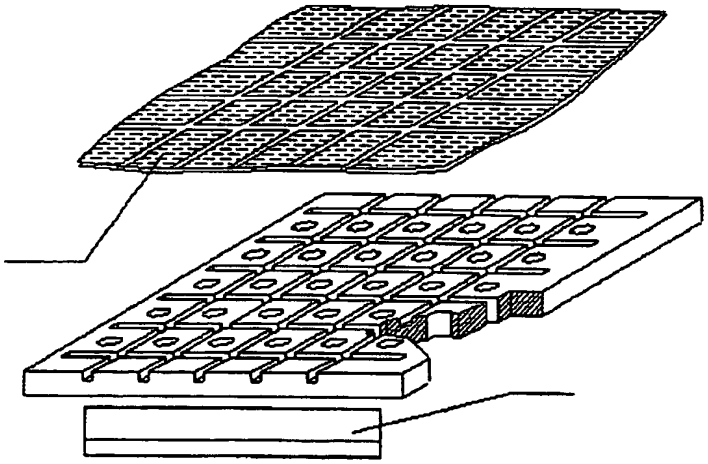


FIG. 3B



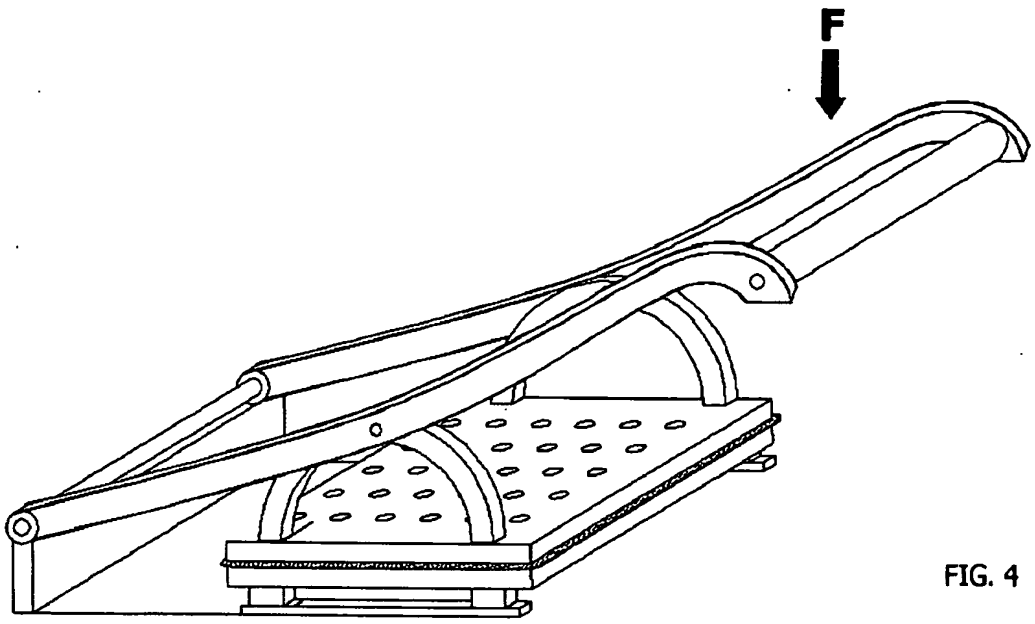


FIG. 4

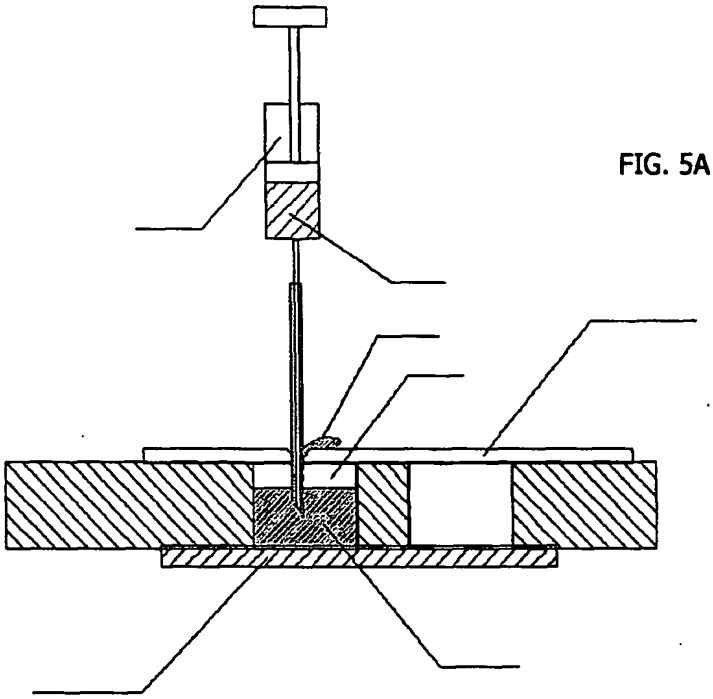


FIG. 5A

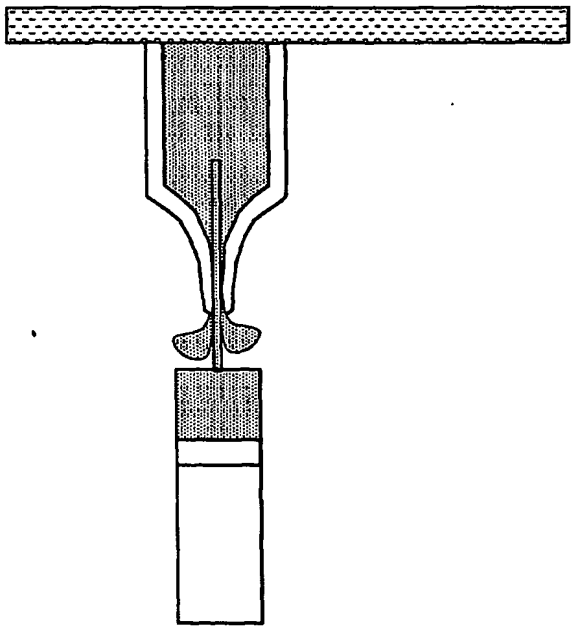


FIG. 5B

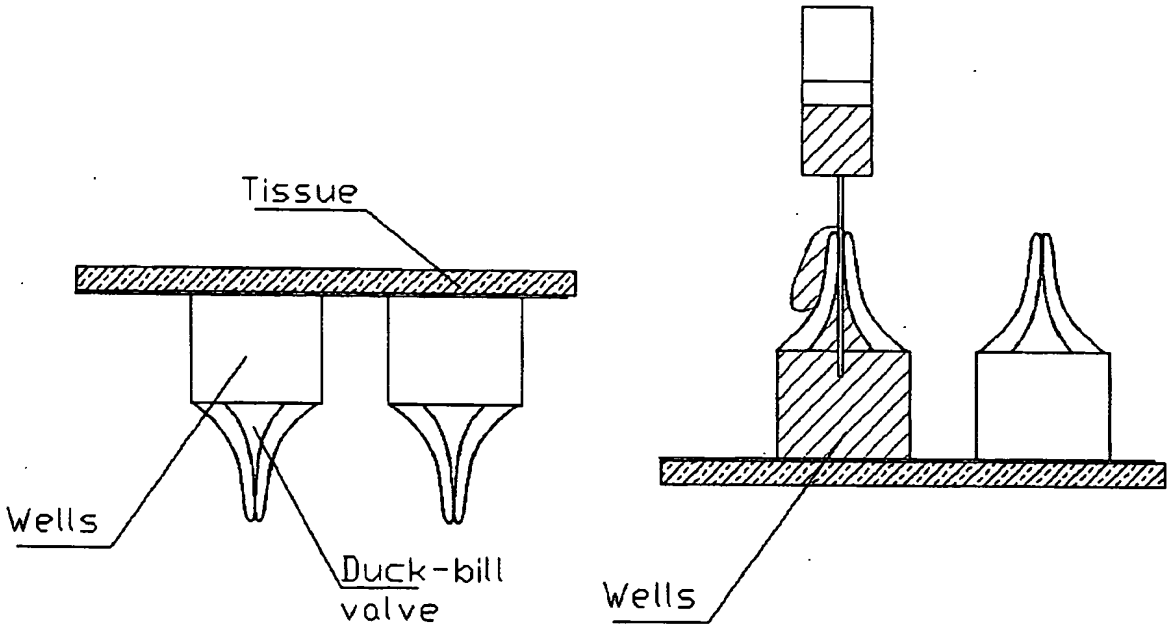


FIG. 6

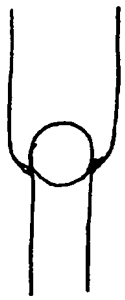


FIG. 7A

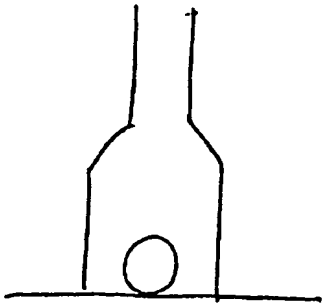


FIG. 7B

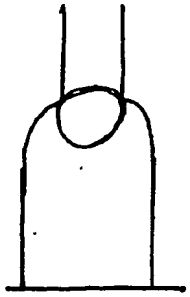


FIG. 7C

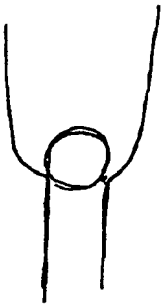


FIG. 7D

FIG. 7I

FIG. 7E

FIG. 7J

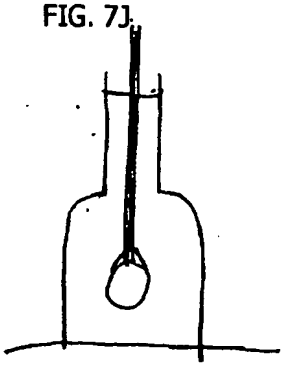


FIG. 7F

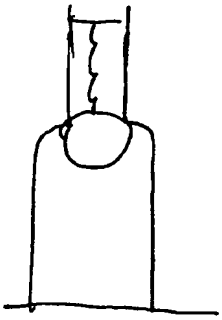


FIG. 7G

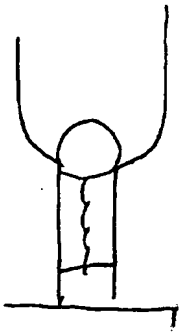


FIG. 7H

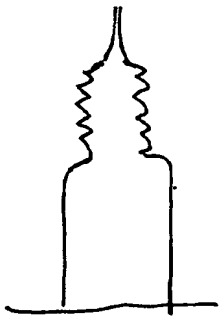


FIG. 7I

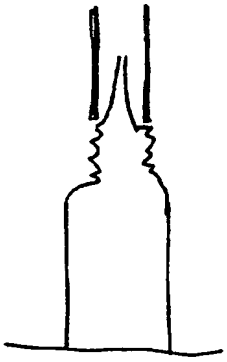


FIG. 7J

FIG. 7

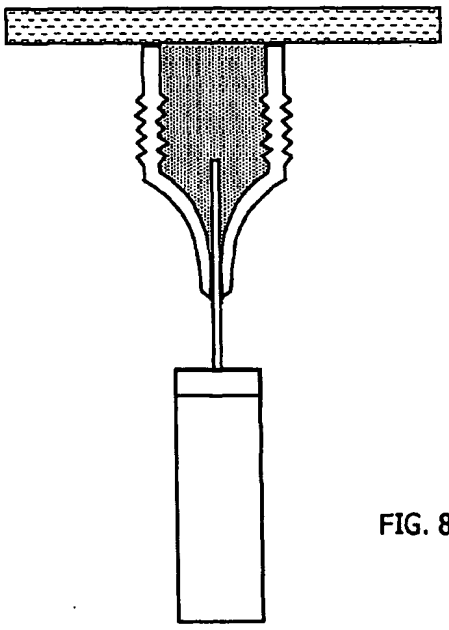


FIG. 8A

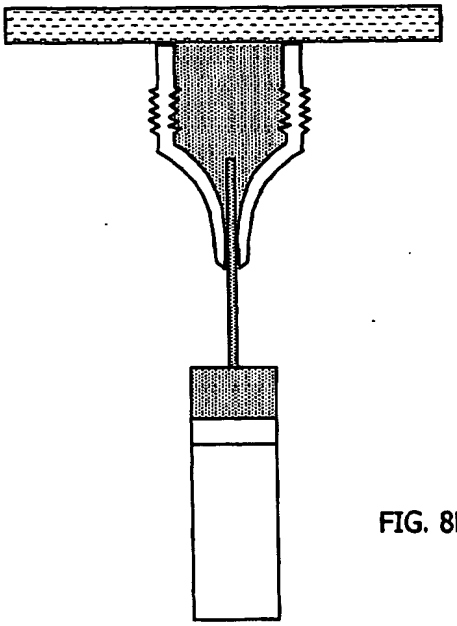


FIG. 8B

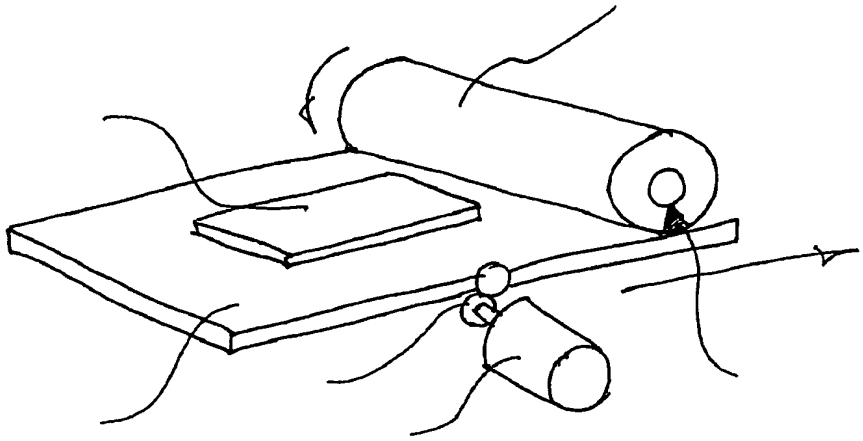


FIG. 9

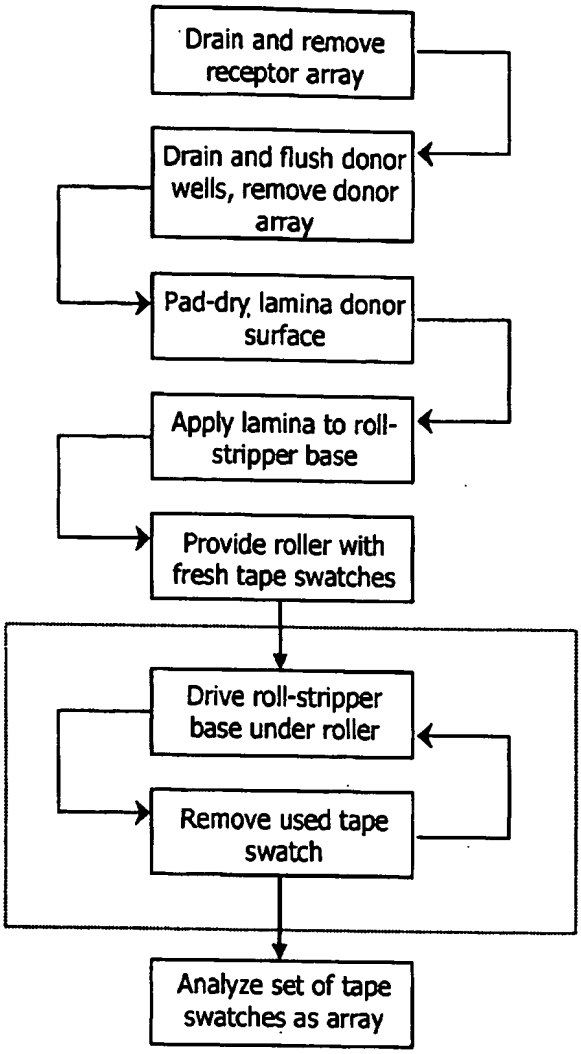

Drain and remove receptor array
Drain and flush donor wells, remove donor array
Pad-dry lamina donor surface
Apply lamina to roll-stripper base
Provide roller with fresh tape swatches
Drive roll-stripper base under roller
Remove used tape swatch
Analyze set of tape swatches as array

FIG. 10

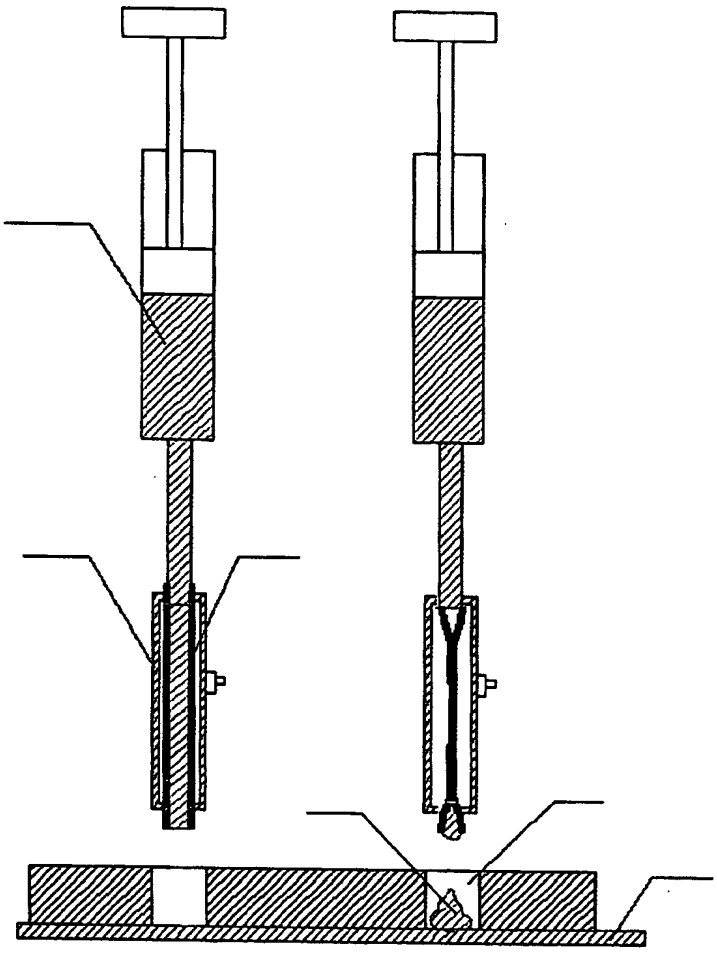


FIG. 11

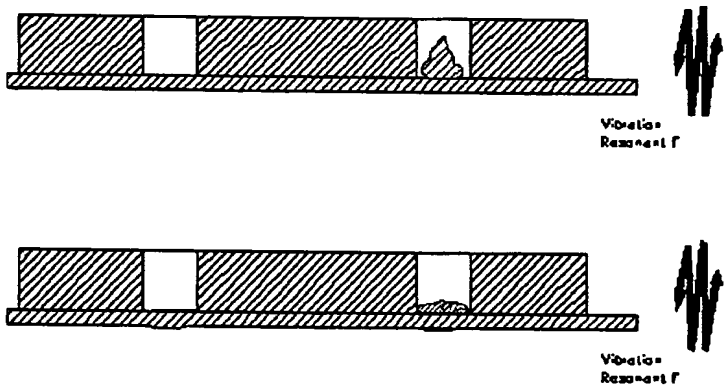


FIG. 12

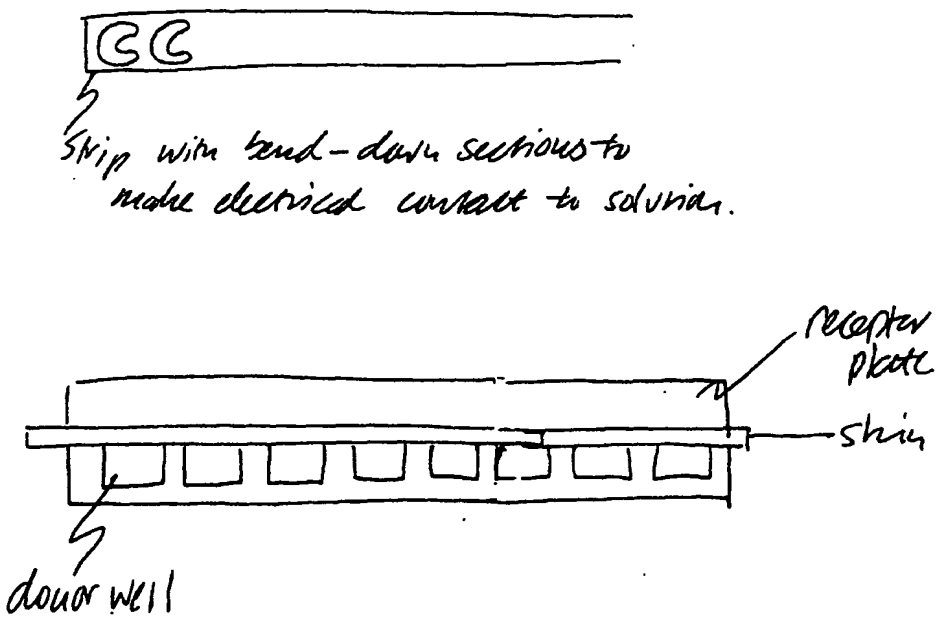
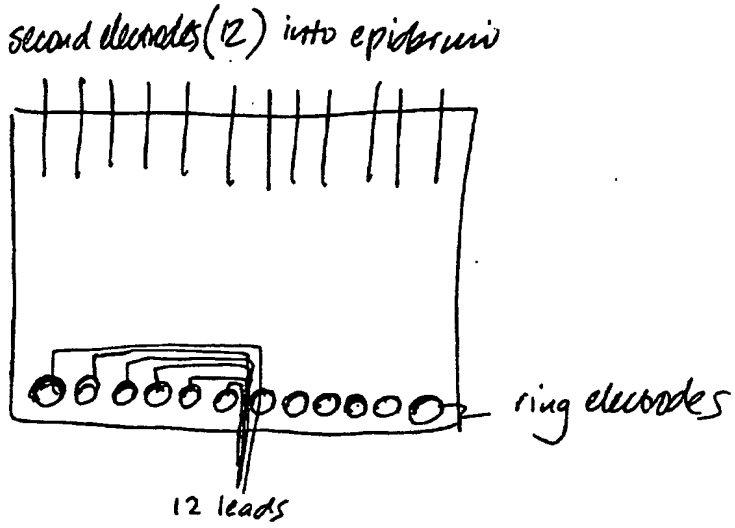

CC
Strip with sand-down sections to make electrical contact to solution.
receptor plate
strip
donor well
8x12 array of donor wells
Slices through strip preferably by ceramic blade That remains in place

FIG. 13


second electrodes (12) into epidermis
ring electrodes
12 leads

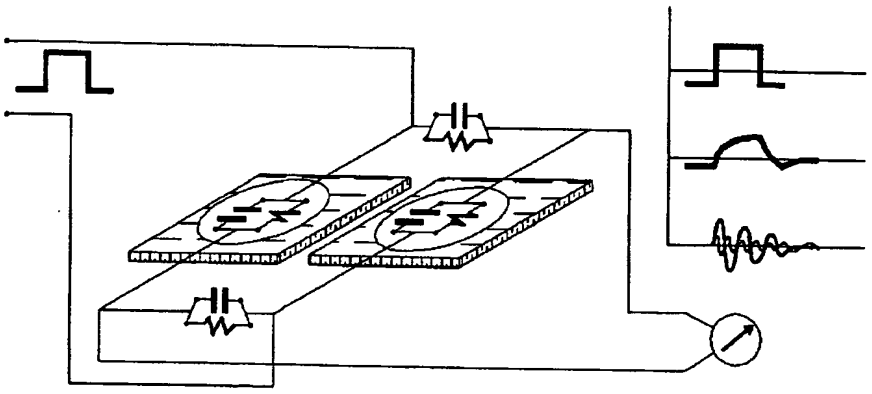


FIG. 14

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/024760

International filing date: 30 July 2004 (30.07.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/491,553
Filing date: 01 August 2003 (01.08.2003)

Date of receipt at the International Bureau: 16 September 2004 (16.09.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse